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Proceedings
of the
Indiana Academy
of Science

Part I
The Work of the Academy

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INDIANA ACADEMY OF SCIENCE
SPRING MEETING OF THE EXECUTIVE COMMITTEE

April 10, 1987

MINUTES

President Stanley Burden called the meeting to order at 2:00 p.m. in the Oak Room, Canyon Inn, McCormick's Creek State Park. All members were present, and there were several guests.

Minutes of the Fall 1986 meeting of the Executive Committee were approved.

Duvall Jones, speaking for the Logo Committee, presented the logo suggested by James Gray of Stone Cabin Design. After some discussion of the design, it was moved and voted that the matter be returned to the committee for further study.

Several miscellaneous items were approved for presentation to the Council.

Frank Guthrie, Chair of the Academy Foundation Trustees, reported that the Trustees recommend new policies concerning income derived from the sale of monographs, and establishment of a new administered account to be known as *Administrative Reserves*. After considerable discussion Alfred Schmidt moved the adoption of the policies, to be presented by the Trustees as a motion from the Executive Committee to the Council. (See Council minutes.)

The meeting was adjourned at 3:00 p.m.

Respectfully submitted,
Richard L. Conklin, Secretary

INDIANA ACADEMY OF SCIENCE
SPRING MEETING OF THE COUNCIL

April 10, 1987

MINUTES

President Stanley Burden called the meeting to order at 3:15 p.m. in the Oak Room, Canyon Lodge, McCormick's Creek State Park. About 25 people were present.

Minutes of the Fall 1986 meeting of the Council were distributed and approved.

TREASURER'S REPORT

Treasurer Duvall Jones reported the following:

	Academy Accounts	Administered Accounts	Total
Balance: January 1, 1987	$14,588.94	$23,073.05	$37,661.99
1987 Income	6,834.18	8,239.12	15,073.30
1987 Expenditures	8,614.14	3,155.05	11,769.19
Balance: April 17, 1987	$12,808.98	$28,157.12	$40,966.10

Trust Funds

Anticipated income and expendable funds as of January 1, 1987:

IAS Fund	$ 2,900.00
John S. Wright Fund	41,000.00
Invested Income Account	131,00.00

Approved expenditures:

Research Grants	$ 35,600.00
Fiduciary Fees	7,00.00
Publications	76,800.00
	$119,400.00

Membership:

Paid for 1987	523
On file not paid	619
New and reinstated	60

The report was adopted.

President Stanley Burden presented several items:

The William A. Wilson Education Center is requesting support for a program designed to stimulate creative thinking in school children. The Academy has its own program for school children, which is under-funded, so no funds are available for other programs. Correspondence with Ball State University concerning the name of a proposed Academy of Sciences, Arts, and Humanities has brought assurance that the name will be modified to avoid confusion with the Indiana Academy of Science.

Ten of the twelve regional science fairs have requested funds from the Academy.

REPORTS OF ELECTED COMMITTEES

Academy Foundation Trustees (Frank Guthrie, Chair)

An unaudited summary of transactions and holdings was distributed.

The following proposals were presented for adoption, as a motion from the Executive Committee.

a. Income derived from the sale of monographs or other publications which were produced by funds obtained from the Invested Income Account will be reinvested in that account.

b. The Trustees will, on an annual basis, transfer appropriate funds from the Invested Income Account to a new administered account, under the supervision of the Treasurer, to be known as Administrative Reserves. The actual amount will depend upon both needs and resources and will be reported to the Budget Committee prior to completion of the annual budgeting process. These funds may be expended by the Executive Committee, as required for the general expenses of the Academy which are incurred in relation to research activities.

The Trustees will maintain adequate reserves to insure an orderly cash flow and emergency funds through a balanced investment program intended to assure preservation of capital, good growth potential and reasonable income to support the eligible programs of the Academy.

Alice Bennett moved acceptance of *a* and *b*.

In discussion two points were clarified: Proposal *a* is intended to put money from sale of monographs back into research activities. "Research activities" may be interpreted to include publication of Symposium brochures. The motion carried.

Research Grants (presented by S. Burden for Austin Brooks)

Fifty two proposals have been submitted, requesting $64,216.00. Decisions are pending on the distribution of the $20,000.00 available.

Delivery of 15,000 copies of a new brochure is expected in the next month.

REPORTS OF STANDING COMMITTEES

Editorial Board (Donald Winslow)

One hundred and ninety papers were presented at the 1986 Fall meeting. One hundred and eighty nine abstracts and 61 manuscripts were received for review for inclusion in the *Proceedings*, Volume 96.

Emeritus Members Selection Committee (Robert S. Cooper, *in absentia*)

Applicants for Emeritus membership are:

Dr. Paul Gebhard, Nashville, Indiana
Mr. Carl H. Krekeler, Valparaiso, Indiana
Mr. Gerald J. Shea, Terre Haute, Indiana

All applicants were approved.

Dr. Frank Edmondson has requested Emeritus rank but his application has not been acted upon. Council recommends that Emeritus rank be granted retroactive to the Butler meeting if he did indeed apply at that time. If not, his recent letter will be recognized as an application to be acted on at the Fall meeting.

Committee on Fellows (Marvin Carmack)

The Committee has been expanded from 12 to 16 members. Each of the 17 Sections of the Academy now has at least one member on this committee. Committee members have been provided with updated lists of Fellows and with nomination forms. The form will also be included in the April *Newsletter*.

The Committee has been requested to consider options for giving more recognition to outstanding scientists who are residents of the state. After some favorable comments on the idea of naming a Hoosier Scientist of the Year, E. Campaigne moved that the President appoint an *ad hoc* committee to study the matter of special awards and report to the Council at its Fall meeting. The motion carried.

Science Talent Search (Donald Winslow)

Forty two students participated in the 40th Annual Indiana Science Talent Search. Nineteen of them were declared Finalists by the Committee and were invited to participate in the Honors Program the weekend of March 6-7 at the Medical Center Union Building, Indianapolis. The activities of the search were funded by Kappa Kappa Kappa, Inc. ($2,500) and by Public Service Indiana, Inc. ($1,000). In addition to these, Ann Koons of East Noble High School, Kendallville, and Brian Worrall of Terre Haute South Vigo High School each was awarded by Kappa Kappa Kappa a $1,000 scholarship.

Invitations (Marshall Cady)

The 1988 meetings will be held April 29-30 and November 10-12 at St. Mary's College. Chancellor Leon Rand of Indiana University Southeast has invited the Academy there in 1989. The Council moved to accept the invitation and set April 14-15 as the dates for the Spring meeting to beheld at Clifty Falls State Park. The Fall meeting at I.U.S. will be November 9-11.

Library (Holly Oster)

Volume 95, (1985) of the Proceedings has been mailed to members of the Academy. Work continues on the mailing of library, exchange, and purchased copies.

Publications (Benjamin Moulton)

Butterflies of Indiana should be available for distribution in September.

The Committee has had a request for financial support of color prints for a book now being published by the Indiana University Press. The Committee's response was that they should not support publication of a volume not reviewed by the Academy or concerning an Indiana topic. Several comments indicated that there might be cases when support for non-Academy publications would be appropriate. Alice Bennett moved that the Publication Committee take into account comments made here and come back in the Fall with a statement of policy on how to deal with publications that do not fit into the standard procedures. The motion carried.

Speaker of the Year (John B. Patton)

Dr. Ruth Howes, of the Department of Physics and Astronomy, Ball State University, is the Speaker of the Year. She has presented her address, "Science and National Security Policy" at the Fall meeting of the Academy, and at several Indiana colleges.

Science and Society (Edwin Squires)

In an effort to increase attendance at its Symposia, the Committee is considering scheduling them from 9:30-4:30 on Saturdays not immediately following Academy meetings. The state museum may become a co-sponsor. Two topics are being considered for next Fall: A salute to Canada, and ground water quality.

Youth Activities (Jan Woerner, Director, Indiana Science Olympiad)

The third annual State Torunament of the Indiana Science Olympiad was held on March 21, 1987, at Center Grove High School, Greenwood. 13 Division B (middle/junior high school) and 16 Division C (senior high school) teams participated. First place winners were the teams from Gavit Middle School, Hammond, and North Central High School, Indianapolis. They are to represent Indiana at the national tournament in Columbus,

Ohio, in May. Jan Woerner will serve as official score keeper for two events in that tournament.

Academy members are needed to help with the organization and operation of the 1988 Science Olympiad.

Plans are underway to have a one-day state symposium in the Fall to introduce about 65 school teachers to Science Olympiad. Funding will be needed for this project, for additional regional events before the state tournament, and to help send the state winners to Delaware for the national tournament. A site may also be needed for the 1988 state tournament.

Corporate Relations (Ernest Campaigne)

This newly constituted committee has two objectives:

An improved plan for recruiting additional corporate members.

Organization of a task force of representatives from corporate members to devise ways of mutual service between such members and the Academy.

Old Business

Executive Secretary Feasibility Committee (Benjamin Moulton)

The Committee met at 1:00 p.m. to consider carefully the need for an Executive Secretary and the functions of the office. There was general agreement that the Academy does not need a paid office manager, but there is a place for a person to provide continuity, increase the profile of the Academy, and find ways the Academy can serve the state. The office is not intended to speak for the Academy, or to become a lobby.

[The meeting was recessed for dinner.]

Benjamin Moulton presented the following motion:

> The Executive Secretary Feasibility Committee recommends that the Academy establish a part-time paid position for a person whose primary responsibilities would focus on researching and coordinating Academy activities, identifying significant projects the Academy should be involved in, developing relationships, and coordinating activities with other scientific organizations within and without the state which will enhance science and promote the Academy. His efforts would be coordinated by the desires and objectives of the Academy Council. The Feasibility Study Committee will pursue the details and job description of such a position and bring forward a formal recommendation to the Fall 1987 Council meeting.

During discussion of the motion questions of funding, of the experience of other state Academies, and some aspects of the job description were considered.

The motion carried.

Duvall Jones presented a progress report from the Logo Committee.

The meeting adjourned at 8:00 p.m., and the evening program began.

Dr. Marshall E. Parks of the Science Teaching Center, Indiana State University spoke to the title "Environmental Education: One Perspective."

Respectfully submitted,
Richard L. Conklin, Secretary

INDIANA ACADEMY OF SCIENCE
FALL MEETING OF THE EXECUTIVE COMMITTEE

November 5, 1987

MINUTES

President Stanley Burden called the meeting to order at 1:35 p.m. in the State Room, Tirey Memorial Union, Indiana State University, Terre Haute. All members were present, and there were several guests.

Minutes of the Spring 1987 meeting of the Executive Committee were approved.

Duvall Jones presented the design recommended by the Logo Committee. E.E. Campaigne moved that it be presented to the Council for adoption. The motion carried. Jones will look into details of having a pin made, and the Secretary will speak to a printer about including the logo on Academy stationery.

Burden announed that Manchester College might be interested in acting as host for the 1990 meeting of the Academy. The matter was referred to the Invitations Committee.

There was a discussion of the need for a policy concerning the presentation at Section meetings of papers for which abstracts have not been submitted. The unwritten policy has been that in order to be presented, a paper should be announced in the printed program, which now means an abstract must be included. Enforcement of this policy is in the hands of the Section chairs. The consensus was that no formal action is needed, but the policy should be explained in the *Newsletter*.

Benjamin Moulton, speaking for the *ad hoc* Executive Secretary Feasibility Committee, presented a proposed motion that the Adacemy appropriate $15,000 per year for the next three years to support the position of Executive Secretary. Alfred Schmidt moved that the Executive Committee accept the recommendation and present it to the Council. After discussion of possible sources of the money, the motion carried.

The Publications Committee was requesting at the Spring meeting to consider revising procedures so that it would be possible for the Academy to provide support for publications from non-Academy sources. Moulton reported that the Committee has not met, but his sense is that it will want to retain control of Academy-funded publications. No further discussion is appropriate until the Committee has studied the matter.

Burden presented on behalf of the Parliamentarian recommendations that all but routine resolutions be submitted in writing to the Committee on Resolutions for processing before being presented for vote. The recommendations were referred back to the source for clarification.

The meeting was adjourned at 2:50 p.m.

The Executive Committee reconvened at 8:15 p.m. to discuss the next steps in the establishment of the office of Executive Secretary.

It was agreed that dues could not be increased in 1988, but that an administered account could be established by the Budget Committee. Several sources of funds were considered. The Executive Committee will meet again on November 28 or December 12 to consider possible applicants for the position, and description of the duties of the office.

Respectfully submitted,
Richard L. Conklin, Secretary

INDIANA ACADEMY OF SCIENCE
FALL MEETING OF THE COUNCIL

November 5, 1987

MINUTES

The meeting was called to order by President Stanley L. Burden at 3:10 p.m. in the State Room, Tirey Memorial Union, Indiana State University, Terre Haute. Thirty members were present.

Minutes of the Spring Meeting of the Council and the Academy were distributed and approved.

TREASURER'S REPORT

Treasurer Duvall Jones distributed his report of finances from January 1 to November 3, 1987. There are 1,058 members, 115 of whom joined or were reinstated during 1987. One hundred and four members and clubs were dropped for non-payment of 1986 dues; 256 have not yet paid 1987 dues.

Summary of finances:

	Academy Accounts	Administered Accounts	Total
Balance: January 1, 1987	$14,588.94	$23,073.05	$37,661.99
1987 Income	14,739.99	55,635.88	70,375.87
1987 Expenditures	14,855.02	49,897.48	64,752.50
Balance: November 3, 1987	$14,473.91	$28,811.45	$43,285.36

Jones announced that his office now has a computer in place for keeping membership and financial records, and thanked Alice Bennett and Ball State University for years of assistance in these matters.

He moved that funds be transferred from Academy Development Fund, Postage and Shipping, and Office Services to Publications to pay the cost of publishing the Program and Abstracts booklet.

He also moved that the dues structure for 1988 be the same as that for 1987.

Both motions carried and the report was approved.

Reports of ad hoc Committees

Logo Committee

Duvall Jones reported that the Logo Committee recommends that the Academy adopt the design on the front of the 1987 Program and Abstract booklet as its official logo. Colors recommended are gold on an Indiana blue field. He moved that the Council accept the recommendations. After some discussion, the motion carried.

Awards Committee

Marvin Carmack reported that he has been gathering information about ways that outstanding individuals can be recognized by the State of Indiana and by other organizations and institutions. The committee will continue to study the matter, possibly polling members of the Academy for their opinions, and will report its progress at later meetings.

Executive Secretary Feasibility Committee

Benjamin Moulton reviewed the history of the committee and actions of the Academy. He circulated a motion recommending the allocation of funds and the hiring of an Executive Secretary, and a list of proposed duties of such an office. In the ensuing

discussion, several issues were raised: Is the expenditure of funds to increase the influence of the Academy in the state proper, or should Academy funds be devoted to the support of research? Can a suitable person be found on a part-time basis, and if so, can the purposes be accomplished in the time envisioned? Can the position be funded with available monies, can the Executive Secretary be expected to increase revenues sufficiently, or must the dues of members be increased? Who will review and direct the activities of the Secretary; will he be autonomous, or governed by the Executive Committee or Council?

The following motion, that of the Committee as re-worded by Jack Leonard, carried with 20 affirmative votes, 2 negative, and 4 absentions:

The Academy shall allocate the sum of $15,000 a year of the next three years to support the position of Executive Secretary of the Academy. The Executive Committee will proceed to fill the position and to prepare a description of the duties of the office, and will report to the Council at its Spring meeting.

REPORTS OF ELECTED COMMITTEES

Academy Foundation

The Indiana National Bank reported that as of October 26, 1987, the Foundation Account has a market value of $49,794. The John S. Wright Fund has a market value of $1,144,673. The Invested Income Account has a value of $133,300.

Research Grants

Wilson Lutz, acting chair, reported that in the Spring 52 applicants had requested $64,650. Twenty projects were funded, in the amount of $20,775. During the summer 13 high school projects were funded, totaling $2,100. In the Fall round of grants, 20 applicants requested $20,770.93, $9,225 was awarded to 16 projects. Lists of awards granted in Spring and Fall are appended to these minutes.

Lutz noted that for the year the difference between requests and grants was $55,724. Even admitting that not all requests deserve full funding, he suggested that an increase of grant money to $35,000 would be appropriate.

Reports of Standing Committees

Only those committees whose reports required action or comment were heard. Other committees submitted written reports which are summarized at the end of these minutes.

Nominations

Alice Bennett presented the names of those to be nominated at the General Meeting. The slate was approved.

Editorial Board

Donald Winslow announced that Volume 96 of the *Proceedings* is promised for January, 1988. The cost of publication will be approximately the same as for the past two years. One hundred and ninety two pages were presented at the Fall, 1987, meeting. In Volume 96, 50 manuscripts and 132 abstracts will be published.

Emeritus

Robert Cooper reviewed the qualifications of four applicants for Emeritus membership and moved that their names be presented to the membership for election. The motion carried.

Fellows

Marvin Carmack reported that the Committee has voted to recommend a record number, sixteen, persons for promotion to the rank of Fellow of the Academy. The list was approved and will be presented to the members at the General Meeting.

Invitations

The President announced that St. Mary's College will be host for the meetings in 1988, Indiana University Southeast for 1989.

Resolutions

William Davies introduced two resolutions, one expressing thanks to the host institution, the other to Ball State University for its years of assistance in record keeping and mailing. Both resolutions were approved for presentation at the General Meeting.

Science and Society

Edwin Squiers commented on the Academy's developing relationship with the Indiana State Museum. The immediate results are symposia on Acid Rain and Ground Water. The first was held in October and was attended by 60 people and covered by Channel 13. The second will be on December 5.

Publications

Benjamin Moulton said that the Committee is considering the issue raised by a request for financial support of the publication of a book written outside the normal Academy controls. He asked for opinions that might guide the Committee's discussion.

Butterflies of Indiana has been released. Copies are available at a reduced rate during this meeting and will go on sale at other outlets soon. William Eberly, editor of *Butterflies*, said he had had a request from a firm in Chicago to use a picture from the book in its advertising. After considerable discussion the following motion, made by Jack Leonard, was approved:

The Academy grants to Ross-Ehlert, Inc., of Chicago, permission to use a specified photograph from *Butterflies of Indiana* for the use specified in their letter to Dr. Eberly, with the stipulation that they acknowledge the source of the photograph and that they be invited to make a donation to the Academy in lieu of usage rights payment.

Other Business

John Bacone voiced his concern about the loss of herbaria from Indiana. He hopes that before biology departments allow these resources to leave the state they will consult the Indiana State Museum.

The meeting was adjourned at 5:50 p.m.

Summaries of Written Reports

Biology Survey (James Aldrich and John Bacone)

The Committee's emphasis is on the rarest elements of Indiana's biota, including encouraging research on community types and natural areas. Among the species being emphasized are bryophytes and several invertebrates.

The Committee was one of the sponsors of the Indiana Natural Areas Conference at New Harmony, which featured a number of presentations about the natural history of southwest Indiana.

Corporate Relations (E. Campaigne)

Members of the Commmittee met with representatives of the five member corporations and a group of Academy officers on Friday, September 25, at the Eli Lilly Corporate Center in Indianapolis. Academy officers described the background of the Academy, followed by a discussion of the place of the Academy in the scientific organization of the state and reasons corporations should support Academy programs. It was agreed that each of the corporations would appoint a representative to the Corporate Relations Committee and this enlarged committee would explore areas of cooperation.

Library (Holly Oster)

Distribution of Volume 95 of the *Proceedings* was completed in July, 1987. A total of 788 volumes were mailed to Academy members, with 65 sent to Indiana academic and public libraries. Paperbound copies were sent to 426 domestic and foreign exchange agencies.

During fiscal 1987, 268 volumes were added to the library's collection bringing the total to 11,475. Three hundred and seven inter-library loan requests were processed. Since January the State Library has participated in a telefascimile network that allows even more rapid response to inter-library loan requests.

In July, 1987 "advice of allotment" for $8,900 was received from the State Budget Agency, to be applied to the cost of printing Volume 96 of the *Proceedings*.

Natural Resources Commission Representative (Damian Schmelz)

In June a new amphitheater at Lincoln state Park was dedicated. The August meeting studied recurring issues in the areas of deer herd management and water resources rights.

Youth Activities (Virginia Rhodes and John Huffman)

The fall meeting of the Junior Academy, directed by Mark Whitman, will be held concurrently with the Senior Academy's annual meeting on November 6. Seventy-eight schools are expected to be present. IBM is underwriting some of the expenses with a grant of $1,800. Purdue University Schools of Science and Mathematics have donated $1,200 to be used for teacher recognition.

The 41st Indiana Science Talent Search will be held March 4-5, 1988, at the Indiana University Medical Center in Indianapolis under the directorship of Donald Winslow.

Jan Woerner is Director of the Indiana Science Olympiad, to be held on March 12, 1988, at North Central High School in Indianapolis for middle and high school students.

The third annual Hoosier Science and Engineering Fair will be held April 14-16, 1988, at the Children's Museum in Indianapolis. Entrants are winners of the twelve science fairs around the state to which the Academy contributed $3,000.

A grant of $1,400 from the AAAS helped support high school science research projects. Proposals were evaluated by the Research Grants Committee of the Academy.

Respectfully submitted,
Richard L. Conklin, Secretary

INDIANA ACADEMY OF SCIENCE
Spring, 1987, Grant Applications Funded

RESEARCH GRANTS COMMITTEE

No.	Principal Investigator/ Institution	Title	Award
1	Simon Beeching Biology IU-Bloomington	The Role of Visual Cues in the Reproductive Behavior of the Cichlid Fish	$ 400.00
2	Robert DiSilvestro Foods & Nutrition Purdue	Influence of Thyroid Hormone on Cu-Zn Superoxide Dismutase Levels...	1,800.00
3	Larry Ganion Physiology and Health Ball State University	Construction of a cDNA Library for Further Study of Annulate Lamellae	1,000.00
4	Charles Hammond Biology St. Meinrad	Characterization of Specialized Plastids in the Glandular Secretory System...	1,800.00
5	David Hirt (Gary Lane) Geology IU-Bloomington	Spiriferid Brachiopod Biostratigraphy and Depositional History of the Ramp Creek Formation...	275.00
6	Daryl Karns Biology Hanover	Analysis of a Hybrid Zone between Northern and Southern Leopard Frogs in SW Indiana	1,000.00
7	Kathleen Kolberg Biol. Sci. Notre Dame	A Fine-Structural Study of the Developmental Toxicity of Nicotine in Halocordyl disticha	400.00
8	Olga (John Dumna) Biol. Sci. Notre Dame	Behavioral and Physiological Adaptations to Cold in a Freeze-tolerant Artic Insect...	800.00
9	Kay McCrary Biology IU-Bloomington	Field Guide to Indiana Wildflowers	1,100.00
10	Eric Menges Holcomb Butler	Vegetation, Environment and Fire in a Post Oak Flatwoods Barrens...	650.00
11	Eric Menges Holcomb Butler	Population Biology of Silence regia in Indiana	1,800.00
12	Scott Mills Animal Science Purdue	Influence of Beta-adrenergic Agonists on Lysine Requirement...	1,000.00
13	Catherine Mondloch (Wm. Rowland) IU-Bloomington	A Description and Experimental Analysis of Parental Feeding in Pigeons	400.00
14	Dorothy Morre Foods & Nutrition Purdue	The Effect of Vitamin A on Golgi Apparatus Functions	1,750.00

15	John Munford Biology Wabash	Hormonal Regulation of Sodium Transport in Rat Skeletal Muscle	1,800.00
16	Robert Olsen Chemistry Wabash	Nucleophilic Substitution/Fragmentation Reactions of Trimethylene Sulfites	1,000.00
17	George Parker Forest Ecol Purdue	Long-Term Research in Forest Ecosystems	1,600.00
18	Robert Pinger Pub. Health Ent. Ball State University	Comparative Bionomics of Containe Breeding Mosquitoes of East Central Indiana	850.00
19	Mark Schurr Anthropology IU-Bloomington	The Use of Nitrogen and Fluoride Analyses to Develop a Chronology...	350.00
20	Carolyn Vann Biology Ball State University	Regulated Gene Expression in Cyanobacteria	1,000.00
		TOTAL	$20,775.00

INDIANA ACADEMY OF SCIENCE
Summer, 1987, High School Student Research Appliation Funded

No.	Name/School/Sponsor	Title	Award
1	C. David (D. Handy) Harrison W. Lafayette	A Study of the Effects of Stress on Student Performance...	$ 170.00
2	Lori DePew (V. Rhodes) E. Noble Kendallville	Determination of the Effects of Second-hand Smoke...	125.00
3	Jamie Gillette (P. Zeck) Northwestern Kokomo	The Absorption of Selenium by Crambe and Soybean Plants	100.00
4	Dave Kantowitz (D. Handy) Harrison W. Lafayette	Design and Construction of a Computer-Assissted...Crystalizer	200.00
5	Sarah Ley (H. Sollenberger) Cnt. Noble Kendallville	The Effects of Dietary Fish Oil on Aortic and Pulmonary Atherosclerosis	250.00
6	Arnold Ma (S. McConnell) Northside Muncie	An Investigation of the Toxic Effect of Tobacco Smoke on Cell Growth...	200.00
7	Amy Maxson (V. Rhodes) E. Noble Kendallville	The Effect of Low-Level Parental X-irradiation on Development of Rats	100.00
8	Kevin Nickels (D. Handy) Harrison W. Lafayette	The Modification and Interfacing of a D.C. Servo-driven Robotic Arm...	200.00
9	Seth Payton (W.T. Anderson) Paoli Paoli	Pseudomonas putida as an Ice Nucleating Device in Commercial Snowmaking	180.00
10	Ida Primus (N. Longenecker) Adams South Bend	The Possible Effects of Chronic Audiogenic Stress on Ethanol Preference...	100.00
11	Lisa Primus (N. Longenecker) Adams South Bend	Possible Effect of Aspirin on Atherosclerotic Factors...	100.00

12	Elena Shaw (D. Handy) Harrison W. Lafayette	An Investigation of the Gene, LCK, and Its Involvement in Human Leukemia	175.00
13	Briana Swain (D. Handy) Harrison W. Lafayette	Development of a Cancer Cell-Specific Immunotoxin	200.00
		TOTAL	$2,100.00

INDIANA ACADEMY OF SCIENCE
Fall, 1987, Grant Applications Funded

No.	Principal Investigator/ Institution	Title	Award
1	In-Ho Choi George Bakken Life Sci. In. St.	The Effect of Temperature on Bird Muscle Contractility in vivo during Development	$ 400.00
2	Elizabeth David Robert DiSilvestro Food & Nutrition Purdue	The Relationship of Macrophage Superoxide Dismutase Levels to Superoxide Production	875.00
3	Patrick Herendeen Biology Indiana University	Fossil Leguminosae from the Eocene of Kentucky and Tennessee	405.00
4	Jane Koska Biology Indiana University	Ecological Aspects of Reproductive Isolation between Toads...	260.00
5	Hossaine Laghaie William Farquhar Radiation Onco I.U. School of Medicine	The Effect of In Vivo Whole Body Hyperthermia on White Blood Cells	495.00
6	Michael Lee Alice Bennett Biology Ball State	Effects of Dietary Fatty Acids on Murine Mammary Epithelial Cells...	1,000.00
7	David Lipke Douglas Duff Biology I.U.-South Bend	Localization of Angiotensin II Binding Sites in Select Tissues of the Rainbow Trout	360.00
8	Julie Olsen Chemistry Wabash College	Degradation of Long Chain Fatty Acids in Germinating Elm, Rape and Corn	520.00
9	Thomas Platt Biology Notre Dame	Identification of the Moluscan Intermediate Hosts of the Meningeal Worm...	425.00
10	Charles Schauf Biology IUPUI	Patch-Clamp Studies of VICIA FABA Guard Cell: Protoplast	800.00
11	Doreen Short W. Yea Chooi-Odle Biology Indiana University	Structure and Regulation of Drosophila Ribosomal Protein Gene L12	1,160
12	Ann Lou St. Amand Stephen Carpenter Biological Science Notre Dame	Species-specific growth and loss within metalimnetic algal populations	465.00
13	Joseph Thompson Chemistry Ball State	Zinc Speciation in Biological Materials	525.00

14	David Thorne Douglas Anderson Forest & Natural Resources Purdue	Nitrogen Dynamics and Nutritional Ecology of the Plains Pocket Gopher	550.00
15	Scott Watkins Stefan Surzycki Biology I.U.-Bloomington	Characterization and Sequencing of cDNA Clones for the Largest Subunit of Human RNA Polymerase	515.00
16	Harold Zimmack James Plye Biology Ball State	Can Insect Pathologists Control Destructive Insects?	380.00
		TOTAL	$9,225.00

INDIANA ACADEMY OF SCIENCE
GENERAL MEETING

November 6, 1987

MINUTES

The meeting was called to order by President Stanley Burden in Tilson Music Hall, Indiana State University, Terre Haute.

Dr. Richard G. Landini, President of Indiana State University, welcomed the Academy to the campus.

Dr. John E. Oliver, Associate Dean of Arts and Sciences, ISU, in welcoming remarks spoke of the importance of the sciences at Indiana State, and the long relation between the Academy and the University.

Fay Kenoyer Daily, Necrologist, reviewed the lives of nine members whose deaths became known in 1987:

Gerald L. Alexander
Harold Delos Caylor
M.H. George DeKay
Herman Andrew Dettwiler
Robert H.L. Howe
Vivian Annabelle Johnson
Ralph Louis Edwin Seifert
Iva Miriam Spangler
William Arnold Willer

A minute of silence was observed in their memory.

Secretary Richard Conklin reviewed actions taken and reports given at the meeting of the Council of the Academy.

Treasurer Duvall Jones reported that the Academy's finances are sound. He also announced that the Academy now owns a MacIntosh computer upon which records are being kept and from which membership lists and mailing labels can be obtained. He thanked the Ball State University Computing Services for their years of providing computer support, and Alice S. Bennett for acting as the Academy's contact at Ball State.

Marvin Carmack, Chair of the Committee on Fellows, presented names of the following nominees:

Mohammad Behforouz, Professor of Chemistry, Ball State University
Charlotte Boener, Professor of Science Education, Indiana State University
William J. Brett, Professor and Chairman, Department of Life Sciences, I.U.S.
Larry Ganion, Professor, Physiology and Health Science, Ball State University
James D. Haddock, Associate Professor of Biology, I.U.P.U.-Ft. Wayne
Eric R. Johnson, Associate Professor of Chemistry, Ball State University
R. Douglas Lyng, Associate Professor of Biological Sciences, I.U.P.U.I.-Ft. Wayne
Clyde Raymond Metz, Professor of Chemistry, College of Charleston, S.C.
Theresa A. Nagy, Associate Professor and Chairperson, Dept. of Physics, I.U.S.
David R. Ober, Professor of Physics and Astronomy, Ball State University
Robert E. Pace, Professor of Anthropology, I.S.U.

Marshall E. Parks, Professor of Science Education, I.S.U.
Ronald L. Richards, Curator of Natural History, Indiana State Museum
John Richard Schrock, Assistant Professor of Science Education, Emporia State University, Kansas
Stanley S. Shimer, Professor of Science Education, I.S.U.
Carl E. Warnes, Professor and Chairman, Department of Biology, Ball State University

All sixteen were elected Fellows of the Academy.
The Secretary read the report of the Committee on Emeritus members, presenting the following applicants:

William H. Bessey, Indianapolis
Frank K. Edmondson, Bloomington
Robert E. Wise, Fort Wayne
Bernard S. Wostmann, Notre Dame

All were elected Emeritus members.
Alice Bennett, Chair of the Nominating Committee, presented the following slate:

President-Elect	Charlotte M. Boener
Treasurer	Duvall A. Jones
Editor of Proceedings	Donald R. Winslow
Director of Public Relations	Alfred R. Schmidt
Member, Academy Foundation	Frank Guthrie
Member, Research Grants	Joan Esterline Lafuze

She moved election of the nominees. The motion carried unanimously.

William Davies, Chair of the Committee on Resolutions, submitted the following resolutions, which had been approved by the Council:

WHEREAS: The members of the Indiana Academy of Science are deeply grateful to the Indiana State University for the invitation to hold the 103rd annual meeting on the Indiana State University campus; and

WHEREAS: The administration, faculty, staff and students alike have cooperated admirably in providing their facilities and support for this 103rd annual meeting of the Indiana Academy of Science; be it

RESOLVED: That the Academy members here assembled express their sincere appreciation to Dr. Richard G. Landini, President of Indiana State University, for all the courtesies that have been extended to the Academy during this meeting. We are especially grateful to Dr. Charlotte M. Boener, chairperson of the Local Planning Committee, to the members of the committee, and to other facilitators for the arrangements of the entire program and the comfort and conveniences provided the Academy members and guests. We also express our sincere thanks to all Academy members who organized and participated in all aspects of this 103rd annual meeting.

WHEREAS: The University Computing Services of Ball State University since 1975 have provided computer support for maintaining

the Indiana Academy of Science membership rolls and have supplied address labels and dues statements for the Academy, and

WHEREAS: Barbara Stacey, Senior Job Control Language Specialist for the aforesaid University Computing Services, has served in liaison with the Academy for many years and has been helpful, understanding, and patient in managing this large work volume at somewhat unpredictable times, thereby greatly assisting the Secretary in the performance of his duties; be it

RESOLVED: That the Indiana Academy of Science express to Ball State University and to its University Computing Services, particularly to Barbara Stacey, our sincere thanks and appreciation for many years of assistance and support rendered to the Academy and its Secretary.

Announcements:

Jan Woerner, Director of the Indiana Science Olympiad, needs volunteers to help operate and judge the 1988 Olympiad, March 12.

Today is National Chemistry Day.

The Science and Society Committee is sponsoring a Symposium on Indiana's Ground Water, at the Indiana State Museum on Saturday, December 5.

The business meeting was adjourned at 2:00 p.m.

Speaker of the Year Samuel A. Rhine, Director of the Genetic Education Center, spoke on the topic, "The New Genetics: Application and Implications for Our Future."

Respectfully submitted,
Richard L. Conklin, Secretary

INDIANA ACADEMY OF SCIENCE
MEETING OF THE EXECUTIVE COMMITTEE

December 12, 1987

Minutes

Present: Stanley Burden, Wilton Melhorn, Duvall Jones, Richard Conklin, Donald Winslow, Ernest Campaigne, Charlotte Boener.

Stanley Burden called the meeting to order at 1:00 p.m. in the Auditorium of the Indiana State Library.

William Craig of First Departmental Services, Rensselaer, described ways his firm could perform some of the functions anticipated in the Executive Secretary.

The Publications Committee was charged at the Spring meeting to consider possible cooperation with university presses. Because of the need for action before the Spring meeting, the President asked for discussion here. The following motion was approved:

> The Indiana Academy of Science, through its Publications Committee, will consider any request from a university press regarding publication of any appropriate scientific work provided that the request is specific and that the Publications Committee has opportunity to review the work prior to its publication. Recommendations resulting from such considerations will be submitted to the Executive Committee for further action.

Campaigne moved that the Executive Committee accept the offer of John Gallman of Indiana University Press to list Academy publications in the catalog of IU Press, to be sold on a non-exclusive consignment basis. The motion carried.

The Committee accepted the following wording for the job description of the Executive Liaison Officer, for presentation to the Council at the Spring meeting.

Title of Proposed Position

Executive Liaison Officer

Duties

Preamble – Although a variety of aims and goals might be defined by a committee, ultimately the success of the office must depend largely on the interests and capabilities of the appointee working in harmony with the Academy Executive Committee. The final success of the appointment can be measured in:

a. An increase of IAS membership.
b. A more positive public image.
c. An increase in monetary contributions which improve and expand Academy programs.

1. Promote the aims, interests and goals of the Academy as it is part of the science fellowship of the state in regional and local affairs.
2. Identify worthy activities of state and regional organizations and facilitate participation of the IAS where appropriate.
3. Aid in writing proposals and making contacts seeking funds to support Academy activities and interests.
4. Identify and facilitate the role of the Academy in support of museums, education, youth activities, conservation and public TV.
5. Provide continuity and efficiency in the execution of Academy business from year to year.

a. Be responsible for publishing and mailing *Program and Abstracts* for fall meeting in conjunction with Program Chairman.
b. Assist the incoming President in the selection of committee chairs.
c. Develop, update and administer a "Manual of Operations" for Academy officers and section chairs.

After approval of the job description, the position will be announced and candidates sought.

The meeting was adjourned at 4:05 p.m.

Respectfully submitted,
Richard L. Conklin, Secretary

INDIANA ACADEMY OF SCIENCE
BUDGET COMMITTEE

December 12, 1987

Minutes

The meeting was called to order by President-Elect Wilton Melhorn at 9:10 a.m., Saturday, December 12, 1987, in the Auditorium of the Indiana State Library.

Members present and their areas of interest were Stanley Burden (President), Wilton Melhorn (President-Elect), Duvall Jones (Treasurer), Richard Conklin (Secretary), Donald Winslow (Editor), Charlotte Boener (1988 President-Elect), Richard Jensen (1988 Program Co-chair), E. Campaigne and Max Marsh (Academy Foundation), Virginia Rhodes and John Huffman (Youth Activities), Edwin Squiers (Science and Society), Wilson Lutz (Research Grants), Benjamin Moulton (Publications), Holly Oster (Library).

Minutes of the 1986 meeting of the Budget Committee were approved.

Duvall Jones distributed copies of a statement of financial policies of the Academy, a list of deadlines, and a new form for requesting payments from Academy accounts. He also made available copies of a proposal from Balfour, Inc., for producing pins using the new Academy logo.

Richard Conklin circulated for comment two suggested stationery designs using the new logo.

Treasurer Duvall Jones presented the proposed budget for 1988. The final budget is appended to these minutes. Discussion leading to changes in line items is summarized below.

In keeping with an action of the Council of its Spring meeting, $4,000 from sale of publications were deposited in Foundation accounts. By a vote of two-thirds of its members (Marsh, Campaigne) the Foundation Committee directed that those funds be transferred directly to the Academy and listed as 1988 Income.

The allocation for expenses of the AAAS Representative was increased from $350 to $500 becaue of the increased cost of travel and accommodations.

Because the Biological Survey had funds remaining from 1987 which would cover its major anticipated expenses, its allocation was reduced to $100 for support of a Natural Areas Conference. The Committee will be asked to report on the reason for its support of this conference to justify allocations in 1989 and beyond.

In 1987 there were to be six issues of the *Newsletter*. The appearance of only three seriously inconvenienced the Program and Publications efforts. Because part of the problem was delays in printing by the Rose-Hulman print shop, the allocation to the *Newsletter* was increased to $1,800 so that four issues could be printed commercially. The necessity of obtaining a Bulk Mailing Permit for the Academy was discussed at some length and will be studied by appropriate officers.

Because an action of the Academy in the Spring Meeting requires profits from sales of publications to be included in the Foundation accounts, a new line item of $400 is opened for Publications Committee incidental expenses.

Virginia Rhodes described the successes of various aspects of the Youth Activities work of the Academy to justify continued support of the Science Olympiad, Youth Activities, and Junior Academy budgets.

Jones explained that the Academy Development item includes such things as expenses of the Corporate Relations Committee, the Directory, logo, Treasurer's supplies, etc.

The item for Programs and Abstracts is primarily for mailing the booklets.

Edwin Squiers reported that the Science and Society symposia are being very well

received. The Committee is now attempting to make available in printed or audio form the proceedings of the symposia. Squiers also hopes to acquire a display board so that the Academy can be represented in exhibits sponsored by other science organizations in the state.

From Academy Accounts the discussion moved to the Trust Fund Accounts.

Upon indication that it would be acceptable to the Trustees of the Foundation, Moulton moved that the line item for Executive Secretary in the Academy Accounts be raised to $15,000. The motion carried.

To implement this item, $11,000 must be transferred from the Invested Income Account. In keeping with Constitution requirements, this transfer has been approved by majorities of the Foundation, Budget, Executive, and Research Grants Committee, and of the Council.

In order to match an increased grant from the AAAS, the appropriation for research grants for high school students was increased to $1,400, available in the Academy Fund.

Conklin moved that the amount for research grants for senior Academy members be increased to $35,000. After some discussion of the hope that the increase would improve quality and quantity of research projects funded, the motion carried.

Jones moved that the registration fees for the Fall meeting be continued at $5 for members, $7 for non-members. The motion carried.

Moulton moved that the budget for 1988 be approved as modified. The motion carried.

The meeting adjourned at 12:40 p.m.

Respectfully submitted.
Richard L. Conklin, Secretary

INDIANA ACADEMY OF SCIENCE
Financial Report
1 January — 31 December 1987

I. ACADEMY ACCOUNTS

	Receipts	Budgeted	Expenses	Budgeted
Income				
Dues	$15,786.00	$15,000.00		
Contributions	$282.00	$250.00		
Interest	$2,771.68	$2,500.00		
Transfer from Invested Income	$2,987.63			
Reserve Funds		$10,570.00		
Management Expenses				
Academy Development			$883.33	$2,200.00
CPA Fees			$550.00	$550.00
Equipment (computer)			$2,695.00	$3,000.00
Fees and Insurance			$162.60	$250.00
Membership Services	($296.00)		$200.00	$500.00
Office Services			$801.56	$900.00
Office Supplies			$598.45	$700.00
Postage and Shipping			$932.82	$1,700.00
Telephone			$82.90	$100.00
Travel			$316.85	$400.00
Subtotal			$7,223.51	$10,300.00
Program Services				
AAAS Representative			$250.00	$250.00
Biological Survey			$1,700.00	$1,700.00
Editorial Board Awards			$0.00	$300.00
Meeting Program and Abstracts	($1,670.00)			$1,670.00
Newsletter			$900.00	$900.00
Public Relations			$500.00	$500.00
Science and Society Comm.	$821.00		$2,040.58	$1,600.00
Science Education Advis. Comm.			$0.00	$200.00
Science Olympiad			$265.88	$300.00
Speaker of the Year			$700.00	$700.00
Youth Activities Comm.			$2,678.55	$3,000.00
Subtotal			$9,035.01	$11,120.00
Transferable to Admin. Accts.				
Junior Academy	($2,000.00)	($2,000.00)		
Library Binding	($1,100.00)	($1,100.00)		
Publications: Mailing	($1,300.00)	($1,300.00)		
Reprints		($2,500.00)		
Totals	$16,282.31	$21,420.00	$16,258.52	$21,420.00

II. ADMINISTERED ACCOUNTS

	1 January Balance	1987 Transfers and Income*	1987 Transfers & Expenditures	31 December Balance
Foundation Acct: Publications	$5,994.78	$4,222.34 (I)	$735.66	$8,781.46
Transfer to Mtg & Prog Acct		($700.00) (T2)		
Foundation Acct: Research Fellows	$149.25	$0.00	$0.00	$149.25
Foundation Acct: Senior Res. Grants	$0.00	$29,900.00 (T2)	$30,075.00	$658.56
Funds returned			($833.56)	
Junior Academy of Science	$4.00	$2,000.00 (T1)	$2,984.55	$819.45
Grant from IBM Corporation		$1,800.00 (I)		
Lilly Library Fund	$7,051.66	$0.00	$44.02	$7,007.64
Library Binding	$3,826.30	$1,100.00 (T1)	$1,556.80	$3,369.50

Meetings and Programs	$1,677.22	$3,780.51 (I)	$6,207.39	$1,620.34
Transfer from Prog & Abstr		$1,670.00 (T1)		
Transfer from Found. Acct: Publ.		$700.00 (T2)		
Publications: Mailing	$555.05	$1,300.00 (T1)	$972.48	$882.57
Publications: Printing	$1,952.89	$10,254.70 (T2)	$10,254.70	$1,952.89
Grant from State of Indiana		$8,900.00 (I)		
Direct transfer to printer		($8,900.00)		
Reprints: Volume 95	$187.36	$3,008.43 (I)	$2,899.70	$677.69
Volume 96		$381.60 (I)		
Research Grants: High School	$300.00	$700.00 (T2)	$2,100.00	$300.00
Grant from AAAS		$1,400.00 (I)		
Science Talent Search	$1,374.54	$82.50 (I)	$2,502.51	$2,454.53
Grant from Kappa Kappa Kappa		$2,500.00 (I)		
Grant from Public Service Indiana		$1,000.00 (I)		
Totals	$23,073.05	$65,100.08	$59,499.25	$28,673.88

*I: Income from external sources T1: Transfer from Academy Accounts
T2: Transfer from Academy Trust funds

III. SUMMARY

	Academy Accounts	Administered Accounts	Total
Balance: 1 January 1987	$14,588.94	$23,073.05	$37,661.99
1987 Income	$16,282.31	$65,100.08	$81,382.39
1987 Expenditures	$16,258.52	$59,499.25	$75,757.77
Balance: 31 December 1987	$14,612.73	$28,673.88	$43,286.61

IV. BANK BALANCES (as of 31 December 1987)

Bank One, Rensselaer, IN: Super NOW Checking Acct #21-0497-4	$5,740.21
Hi-Fi Account #70-032504	$11,704.71
CD #11985	$3,313.60
Bank One, Lafayette, IN: CD #66594185	$5,000.00
Indiana Federal Savings and Loan, Rensselaer, IN: CD #10-0140111	$3,612.76
Lafayette National Bank, Remington, IN: CD #2408862	$2,580.48
CD #302641	$2,630.82
State Bank of Rensselear, IN: CD #100212712	$8,704.03
Total	$43,286.61

V. SUMMARY OF TRUST FUNDS

	Research Account (430-00-0)	J. S. Wright Fund (430-01-9)	Invested Income (430-02-8)
Assets (1 January 1987)			
Cash	$188.19	($4,264.19)	$8,365.29
Tax Value of Investments	$42,123.69	$437,935.94	$125,711.11
Tax Value of Account	$42,311.88	$433,671.75	$134,076.40
Income and Transfers			
Dividends	$581.30	$26,579.30	
Interest	$2,362.11	$23,152.44	$9,116.41
Income from Sales	$7,905.16	$122,632.93	($313.19)
Transfer from Wright Fund			$41,440.24
Miscellaneous Receipts	$219.46		
Expenditures and Transfers			
Fiduciary Fees	($10.82)	($6,520.25)	($37.10)
Transfer to Invested Income		($41,440.24)	

Distributions to Programs	($300.00)		($43,542.33)
Transfer to Research Account		($219.46)	
Balance - 31 December 1987	$53,069.09	$557,856.47	$140,740.43
Assets - 31 December 1987			
Cash	$14.14	($4,755.96)	$4,980.88
Tax Value of Investments			
Short Term Investments	$7,400.00	$20,200.00	$61,000.00
Bonds	$25,742.19	$352,335.45	$74,759.55
Stocks	$19,912.76	$190,076.98	
Tax Value of Accounts - 31 Dec.	$53,069.09	$557,856.47	$140,740.43
Market Value of Principal - 31 Dec.	$50,650.14	$1,263,168.16	$138,699.63

VI. NOTES

Membership. There were 1259 memberships in good standing as of 31 January 1988, including:

5 corporate members
4 honorary members
4 life members
106 emeritus members
46 sustaining members
715 members
145 associate members
133 student members
101 clubs

10 members deceased in 1987 are not included in the totals above.
191 memberships on file from 1986 were not paid in 1987.
174 new and reinstated members joined the Academy in 1987.
104 members and clubs were dropped for nonpayment of 1986 dues.

Dues Structure for 1987:

$5 for clubs and student memberships
10 for associate members
15 for members
25 for sustaining members
300 for life memberships
150-500 for corporate memberships
50-100 for institutional memberships

Reprints: All authors of papers in Volume 95 of the *Proceedings* have paid for the reprints which they ordered. Cost of the reprints to the Academy was $2,899.70. Authors paid the Academy $3,111.50 for reprints.

Publications: Sales of reprints, monographs and *Proceedings* in 1987 netted $6,912.37. More than 1,000 copies of the *Butterflies of Indiana* were sold during the last three months of the year.

Research Grants: Funds totalling $31,341.44 have been awarded to members and students.

Grants Received: The State of Indiana contributed $8,900 toward publication of the Academy *Proceedings*.

The Science Talent Search received $2,500 from Kappa Kappa Kappa Sorority and $1,000 from Public Services Indiana. The IBM Corporation supported activities of the Indiana Junior Academy of Science with $1,800. The American Association for the Advancement of Science granted $1,400 for high school students to use in their research.

The John S. Wright Fund, the Academy's main trust account, supports major portions of the Academy's research grant programs and research-related publications such as the *Proceedings* and monographs.

Administration: Purchase of computer equipment enabled the Academy to centralize its membership services and to computerize its accounting system during 1987

VII. 1988 BUDGET OF THE INDIANA ACADEMY OF SCIENCE

1. Academy Accounts - Income

Dues	$15,500.00
Contributions	$300.00

Interest	$2,300.00
Transfer from Administered Accounts	$4,000.00
Transfer from Investment Interest	$11,000.00
Reserve Funds	$6,100.00
Totals	$39,200.00

2. Academy Accounts - Expenses

Management Expenses	
Academy Development	$1,650.00
CPA Fees	$550.00
Executive Secretary	$15,000.00
Fees and Insurance	$500.00
Membership Services	$200.00
Office Services	$1,100.00
Office Supplies	$650.00
Postage and Shipping	$1,150.00
Telephone	$50.00
Travel	$350.00
Subtotal	$21,200.00
Program Services	
AAAS Representative	$500.00
Biological Survey	$100.00
Editorial Board Awards	$300.00
Newsletter	$1,800.00
Publications Committee	$400.00
Public Relations	$500.00
Science and Society Comm.	$1,600.00
Science Education Advisory Committee	$200.00
Science Olympiad	$400.00
Speaker of the Year	$700.00
Youth Activities Committee	$3,000.00
Subtotal	$9,500.00
Transferable to Administered Accounts	
Junior Academy	$2,000.00
Library Binding	$1,600.00
Meetings: Program & Abstracts	$2,000.00
Publications: Mailing	$700.00
Reprints	$2,200.00
Totals	$39,200.00

3. Trust Funds

Anticipated Income & Expendable Funds (30 September 1987)		
IAS Fund	(00430-00-0)	$3,000.00
John S. Wright Fund	(00430-01-9)	52,000.00
Investment Income Account	(00430-02-8)	132,000.00
Approved Expenditures		
Fiduciary Fees (est.)		$7,000.00
Research Grants for Senior Academy Members		35,000.00
Research Fellowship for High School Teachers		5,000.00
Research Grants for High School Students		1,400.00
Expenses of Research Grants Committee		200.00
Executive Secretary (in part)		11,000.00
Publications		
Program and Abstract Booklet (1988)		1,500.00
Proceedings (Volume 96)		10,500.00
Climate of Indiana		15,000.00
Butterflies of Indiana		35,000.00
Endangered Species		7,000.00
Flora of Indiana		8,000.00
Total		$136,600.00

Respectfully submitted,

Duvall A. Jones, Treasurer

We, the undersigned, have audited the Treasurer's records of the Indiana Academy of Science for the year 1987 and have found them to be accurate and in order.

David D. Chesak

25 January 1988

John Ricketts

24 January 1988

1987 REPORT
YOUTH ACTIVITIES COMMITTEE
INDIANA ACADEMY OF SCIENCE

This committee continues to encourage and promote the development of science knowledge and skills in Indiana's secondary school students through the activities of the Indiana Junior Academy of Science, the Indiana Science Talent Search, the Indiana Science Olympiad, and the state and regional science fairs. Some of the activities also include middle school and elementary students as well.

Mark Whitman was serving his first year as new Director of the Indiana Junior Academy of Science and a major reorganization was undertaken. Because the American Junior Academy of Science/American Association for the Advancement of Science meeting was to be held in Chicago, three student paper presenters were named to attend the national meeting instead of the usual one. They were Ann Koons of Kendallville's East Noble High School, Alvina Ma of Muncie Northside High School and Michael Sacopolus of Terre Haute North Vigo High School. Ed Wuerch of Richmond High School had been named the outstanding delegate at the fall 1986 meeting and also attended AJAS, February 13-17, 1987. Michael Sacopolus was named the Outstanding Junior Scientist of 1986.

The 41st Indiana Science Talent Search was held March 6-7, 1987 in Indianapolis. Director Don Winslow and the Science Talent Search Committee selected eleven students as winners in the competition. Kappa Kappa Kappa, Inc. (Tri Kappa) continued support of the Indiana Science Talent Search in the amount of $2,500. Tri Kappa scholarship winners were Ann Koons, a senior at East Noble High School in Kendallville, and Brian Worrall, a senior at Terre Haute South Vigo High School.

New Director Jan Woerner of Indiana University-Purdue University Northwest organized and ran the Science Olympiad, held on March 31, 1987 at Center Grove High School, Greenwood, Indiana, with teams from eighteen schools participating in Division C (grades 9-12): this division was won by Indianapolis North Central High School. After a full day of various competitions, Hammond Gavit's Middle School was declared the winner in Division B (grades 6-8); 22 schools competed in the latter. Both schools competed in the national meet later in the spring.

Twelve Regional Science Fairs were held between February and April around the state and 24 students were named to be finalists in the 38th International Science and Engineering Fair in San Juan, Puerto Rico in May. The Academy provided financial support to the fairs in the amount of $2,750.

The second Hoosier Science and Engineering Fair was held at the Children's Museum in Indianapolis, April 23-25, 1987 with 72 students participating; six students represented each—two ISEF delegates, two from grades 9-12 who competed to represent the ISEF at the International Fair and two students from grades 7 or 8. Complete funding was obtained from this important event by Direector Karl Kaufman under the auspieces of the Science Education Foundation of Indiana. The two students who attended the ISEF as Hoosier Fair representatives were Deborah Houser, a senior from East Noble High School, Kendallville, and a senior from Bloomington High School North, Michael Terry.

A grant of $1,400 was received as a result of a proposal submitted to the American Association from the Advancement of Science for the support of high school science research projects. The Research Committee of the Senior Academy evaluated proposals from high school students and granted awards in September.

The 55th annual meeting of the Indiana Junior Academy of Science was held Friday, November 6, on the campus of Indiana State University in Terre Haute. Approximately 350 students and their teachers from 78 schools in Indiana participated.

The opening address entitled "The Future Implications of Genetic Engineering" was made by Dr. John Corrigan of Indiana State University. Special tours were made of the ISU campus and Rose Hulman's Center for Applied Optics. A Problem Solving Exam was taken by student delegates while teachers attended a seminar on promoting science research in their schools. Delegates met by regions to discuss: "What are the future implications of genetic engineering, and what must science do to meet its future responsibilities?" Twelve students were candidates for the Outstanding Junior Scientist for 1987, an honor bestowed upon Dragica Balac of Donald E. Gavit High School in Hammond. Her teacher-sponsor was Michael Kobe. Alan Acker of Terre Haute North High School was honored for achieving the highest score on the problem solving exam as were the five top scoring high school teams—Homestead High School of Fort Wayne, Terre Haute North Vigo High School, Fort Wayne Concordia High School, Brebeuf Prep School and Lawrenceburg High School. Twelve students presented papers at the annual meeting:

Dragica Balac Gavit High School Sponsor: Mr. Michael Kobe	"A Study of the Callus Growth and Differentiation of *Daucus carota*, Phase IV: The effects of Electrostatics"
Lori DePew East Noble High School Sponsor: Dr. Virginia Rhodes	"A study of Second-hand Smoke Effects on Murine Metabolism"
Scott Fergusson Central Noble High School Sponsor: Mr. Dan Replogle	"Prosthetic Apparatus"
Rejesh James Homestead High School Sponsor: Mr. Thomas Waning	"The Effect of Radiation on Carrot Clones in the Callus Stage"
Jayma Kern Bedford-North Lawrence High School Sponsor: Mr. Karl Scherschel	"Investigation of Stress Related Situations and Their Psychological and Physiological Responses"
Arnold Ma Muncie Northside High School Sponsor: Ms. Susan McConnell	"A Study of the Relative Toxicity of Second Smoke on Cells Using *in vitro* Cell Culture Techniques"
Robyn Marshal Paoli High School Sponsor: Mr. Tom Anderson	"Investigation of Electrochemical Interactions of Various Metallic Alloys Exposed To An Oral Environment"
Chad Alan Mead Canterbury High School Sponsor: Mr. Don Franklin	"O.C.B.P.C.—Oxygen Creating and Bleeding Pack for Consumption"
Kim Noble Center Grove High School Sponsor: Ms. Carolyn Hayes	"Fetal Alcohol Syndrom: Phase III-Prenatal Effects of Ethanol on Chromosomes"
Ann Nofziger Paoli High School Sponsor: Mr. Tom Anderson	"A Study of Prodigiosin Synthesis in *Serratia marcescens*: Phase III, Using Heat and Specialized Nucleases to Locate the Pigment Cone"
Yvonne Spartz Hamilton Heights High School Sponsor: Mr. Larry Massom	"Investigating the Evolution of Insect Wings"

Michael Syring
Muncie Northside High School
Sponsor: Ms. Susan McConnell

"The Conveyance of Bateria Through Ice Cubes Into Certain Kinds of Beverages"

DePew, James, Ma, Noble and Nofziger were named as finalists and returned to Indianapolis on December 12, 1987 to again present their work to judges at the University of Indianapolis. As a result, Ann Nofziger from Paoli High School was named the 1987 Outstanding Paper Presenter for IJAS. The runner-up was Rajesh James of Fort Wayne's Homestead High School. Both will attend the NJAS/AAAS Meeting in Boston along with Mark Vockell of Hammond Gavit High School who was named the Outstanding IJAS Delegate.

Respectfully submitted,

Virginia Rhodes
Co-chair
Youth Activities Committee

Report from Donald R. Winslow, Director, Indiana Science Talent Search, to:

Youth Activities Committee
Indiana Academy of Science Council

Spring Meeting, McCormick's Creek,
Spencer, Indiana, April 10, 1987

Forty two students participated in the 40th Annual Indiana Science Talent Search. Nineteen of these students were declared *Finalists* by the Committee and were invited to participate in the Honors Program the weekend of March 6-7, 1987 at the Medical Center Union Building, Indiana University-Purdue University at Indianapolis.

The activities of this year's Talent Search were funded by Kappa Kappa Kappa, Inc. at the $2,500.00 level and by Public Service Indiana, Inc. at the $1,000.00 level. In addition to these amounts of monies, Ann Koons of East Noble High School, Kendallville, and Brian Worrall of Terre Haute South Vigo High School each was awarded by Kappa Kappa Kappa $1,000 scholarships which must be used at an Indiana four-year college of higher education. Elizabeth Chilcott, State Philanthrophy Chair, represented Kappa Kappa Kappa, Inc.; and Mike Goss, Educational Services, represented Public Service Indiana, Inc.

On Saturday morning at the *Science Teacher Symposium*, Mark Whitman, Director of the Indiana Junior Academy of Science, shared his views on "Motivating the Talented High School Student." Following his presentation, Duvall Jones, Treasurer of the Academy, presented "New Options and/or New Directions in Biology" to help stimulate thinking about new areas in the life sciences that possibly could be explored by future high school students.

The keynote speaker for the awards luncheon was John D. Hague, President of the Indiana Corporation for Science and Technology. Dr. Hague's address was titled "In Preparation for a Different Tomorrow"—a very suitable topic for our future leaders.

As the Science Talent Search enters its fifth decade, it is appropriate, I think, to refer to a statement made by Ralph Lefler in 1949 at the Executive Committee meeting of the Academy held in Crawfordsville:

> "The necessity for the early identification and for the continued development of our talented in all fields has long been recognized... The rigorous selection procedures which requires an essay on an original project is a maturing and desirable experience for any serious minded high school senior. All Indiana Winners are recommended to the admissions officers and scholarship committees of our Indiana colleges and universities. It is sincerely hoped that no really talented student will have to terminate his program of education because of inability to bear the financial obligation... Personal interviews with students provides opportunity for appropriate encouragement in the field best suited for their continued study."*

These are still part of the objectives of the Indiana Science Talent Search. A list of the Talent Search *Winners* for 1987 is attached.

**Proceedings* of the Indiana Academy of Science, Vol. 59, 1950, pp. x-xi.

40th INDIANA SCIENCE TALENT SEARCH WINNERS
March 6-7, 1987

Beem, Matthew
An Evaluation of the Relative Effectiveness of Different Types of Passive Air Filters: Implications for Respiratory Health
John Adams High School, South Bend
Sponsoring Teacher: Nevin Longenecker

Clark, Brian
A Study of the Effects of (+)-Catechin and A (+)-Catechin- Copper Complex on Metastasis
Harrison High School, West Lafayette
Sponsoring Teacher: Mark Whitman

Cooper, Pamela
Microbiological Implications Concerning the Use of Activated Carbon as point-of-use Water Filtration Device Phase I and Phase II
Muncie Northside High School, Muncie
Sponsoring Teacher: Susan McConnell

Duehmig, Mark
Production of a Radio Telescope
East Noble High School, Kendallville
Sponsoring Teacher: Virginia Rhodes

Kantowitz, David
Design and Construction of a Computer-assisted Non-steady State Crystalizer
Wm. Henry Harrison H.S., West Lafayette
Sponsoring Teacher: Mark Whitman

Kobe, Jeffrey
The Analysis of Water-soluble Internal Combustion Emissions and their Effect on Bateria of the Human Respiratory System
Munster High School, Munster
Sponsoring Teacher: Donald Ullman

Koons, Ann
A Study of the Chemotherapeutic Treatment of Lung Carcinoma *in vitro*
East Noble High School, Kendallville
Sponsoring Teacher: Virginia Rhodes

Nofziger, Anne
Identification of the Biochemical Pathway of Prodigiosin Synthesis Using Color Mutants of *Serratia marcescens* Utilizing Syntrophic Techniques
Paoli Junior-Senior High, Paoli
Sponsoring Teacher: William Tom Anderson, Jr.

Scheel, Frederick
The Possible Prolonged Enhancement of
Physiological Processes with
Vitamin-mineral Supplementation
Following the Cessation of
Strenuous Exercise
John Adams High School, South Bend
Sponsoring Teacher: Nevin Longenecker

Stante, Julie Lynn
Possible Effects of Imposed Stress Due
to Sleeplessness on the Immune
Response of Laboratory Mice
John Adams High School, South Bend
Sponsoring Teacher: Nevin Longenecker

Worrall, Brian
Sensory Applications of Microcomputers:
Digital Speech Recognition and
Vocal Control
South Vigo High School, Terre Haute
Sponsoring Teacher: Melanie Huber

Brian Worrall and Ann Koons were awarded $1,000.00 scholarship by Kappa Kappa Kappa, Inc. These two students must attend Indiana 4-year institutions of higher education in order to validate their awards.

Necrology
Fay Kenoyer Daily, Butler University

Gerald L. Alexander

Winchester, Indiana
December 5, 1906

Fort Myers, Florida
November 25, 1986

Professor Gerald L. Alexander was an assistant professor emeritus of chemistry at Ball State University, who died November 25, 1986, at his home in Fort Myers.

Professor Alexander was born at Winchester, Indiana, December 5, 1906, and was educated through high school there. His advanced studies were taken at Ball State Teacher's College where he received a B.S. degree in 1930 and at Indiana University where an M.S. degree was obtained in 1940.

Gerald Alexander's teaching career began in 1930 after graduating from Ball State Teacher's College. He taught Chemistry at Dunkirk High School and coached basketball, baseball and tennis until 1937 when he moved to the Muncie School. From 1937 to 1943, he taught science at Wilson Junior High School in Muncie.

In 1943, Prof. Alexander became a United States of America Naval Officer in charge of the Seventh Fleet Training Center in the Philippines. After World War II, his assignment in 1946 was the Muncie Trade School, now Area Career Center, to teach radio. In 1947 he joined the Ball State Chemistry Department where he taught for 27 years until his retirement in 1973. He was widely known in science education.

In 1973, he reported that he had been playing tennis for 56 years and intended to play and encourage others to play after retirement. In 1981, he was named to the Delaware County, Indiana, Hall of Fame for his work in tennis as player and coach.

Professor Alexander joined the Indiana Academy of Science in 1974, the next year after retirement from Ball State University. He listed as interests Zoology, Ecology and Entomology. In the *American Men of Science*, the chemistry of rare elements is listed as his chief interest. He was also a member of the American Association for the Advancement of Science, American Chemical Society, the National Teacher's Association, Central Association of Science and Mathematics Teachers and Muncie Technical Society.

Gerald Alexander was considered honests, kind and loving by those who knew him well. He believed he should "do unto others as you would have others do unto you".

HAROLD D(ELOS) CAYLOR

Nottingham, Indiana
August 19, 1894

Bluffton, Indiana
June 16, 1986

Dr. Harold Delos Caylor was a surgeon and at 91 years of age when he died. He was chairman emeritus of the Caylor-Nickel Medical Center at Bluffton, Indiana, which he helped found and where he died June 16, 1986. Dr. Caylor was a native of Nottingham, Indiana, a village in the southeastern corner of Wells County, Indiana, the same county in which Bluffton is located. He was born August 19, 1894, the son of Charles E. Caylor, a rural medical doctor. His grandfather was also a physician.

Dr. Harold D. Caylor attended Pennville High School at Pennville, Indiana, and entered pre-medical school in 1912 at Indiana University. On the south side of Chicago at the University of Chicago, he received a B.S. degree in 1916; and at an affiliate on the west side of Chicago, Rush Medical College, he earned an M.D. degree in 1918. During his senior year, he had done post mortems at the Cook County Morgue and then at Saint Luke's Hospital, commuting to Rush by elevated train during a very cold winter. He developed a cough which persisted and found out that he had tuberculosis. After a rest period for regaining his health, he finished the internship which he had started at Presbyterian Hospital at Evanston, Illinois, as a special student in 1921.

Dr. Harold Caylor's father had visited the Mayo Clinic for 2 weeks a year to observe their methods and after such a visit decided to open a clinic-hospital in Pennville in 1907. By 1921, his practice had moved to Bluffton, Indiana. His son, Harold, joined him as a partner and general surgeon from 1922 to 1924. Harold then joined the Mayo Clinic Foundation as an assistant professor of surgical pathology at Rochester, Minnesota, from 1927 to 1929. It was then back to the Bluffton arrangement to work with his father and brother, Truman, as a general surgeon and partner. The construction on the Caylor-Nickel Clinic Hospital was completed in 1940. It was set up as a 45 bed facility in 1939. Dr. A.C. Nickel, also Mayo trained, had joined the father-son group in 1931. Dr. Truman Caylor's son, Dr. Charles "Chad" Caylor serves now in a leadership role at the hospital. Dr. Harold Caylor wrote a book "A Clinic in a Cornfield—a history of the Caylor-Nickel Clinic and Hospital.".

Dr. Harold D. Caylor served as Chairman of the Board of the Caylor-Nickel Research Foundation, trustee of the Caylor-Nickel Hospital 1922-1975, and member of the executive committee. He retired in 1979 but continued to visit patients in a supportive role even after he was 90 years of age. A tribute in a Center publication called him "a very good physician, leader, friend". Dr. Caylor joined the Indiana Academy of Science in 1931 listing Zoology, Chemistry and Bacteriology as interests. He served as a Division Chairman and was very active in other societies. He was a Fellow of the American College of Surgeons, honorary member of the Minneapolis Surgical Society, past president of the American Association of Medical Clinics, past president of the Indiana Lung Association and Wells County Tuberculosis Association, member of the American, Indiana and Wells County Medical Societies, the American Thoracic Association (pres. 1951-1952), Indiana Bone and Joint Club, Indianapolis Athletic Club, Pan Pacific Surgical Association, Sigma XI and Phi Chi Epsilon. He was founder and past president of the Group Practice Association (now American Association of Medical Clinics) and was active in the "We care" program to help the needy to obtain medical care at a low cost.

Needless to say, Dr. Caylor's many activities and achievements did not go unnoticed. He received a number of awards including an Outstanding Citizen's Award from the Chamber of Commerce in 1980, the United Way Award for Community Service, and he was made a Sagamore of the Wabash.

M. Henry George DeKay

Randolph, Nebraska
August 14, 1898

Lafayette, Indiana
July 9, 1987

A native of Randolph, Nebraksa, Dr. M. Henry George DeKay was born August 14, 1898. He was almost 89 years old when he died July 9, 1987, at Lafayette, Indiana. He was professor emeritus from the School of Pharmacy at Purdue University.

Dr. DeKay went through primary grades and high school in Randolph, Nebraska, and served in the United States of America Army Students Army Training Corps in 1918. He continued with his education at Wayne Teachers College, Wayne, Nebraska, and received a B.A. degree in 1923. In 1928, he received a B.S. degree from the University of Nebraska School of Pharmacy at Lincoln, Nebraska. He spent several summers from 1925 to 1930 at the University of Chicago and finished an M.S. degree in 1930 with a major in chemistry (organic). At Purdue University, he received a Ph. D. degree in 1934. He was a registered pharmacist in Nebraska and Indiana.

Dr. DeKay's teaching career begain as coach of athletics in Tilden, Nebraska, 1920. He was then coach and science teacher in Wausa, Nebraska, in 1921; coach, science and manual training teacher in Stuart, Nebraska, in 1922; coach and Principal of Stuart High School in 1923; and Superintendent of Schools in Stuart from 1923 to 1927. He then left for further work at the University of Nebraska.

Dr. DeKay came to Purdue University in Indiana as an Instructor of Pharmacy in 1928 and moved in rank to Professor of Pharmacy in 1942. He retired as professor emeritus in 1967. His exceptional service at Purdue spanned 39 years during which time he won the prestigious Bowl of Hygea Award in 1960, one of the first in the nation, because of his distinguished service in his profession and community. He was active in the civic affairs of Lafayette in addition to his scholarly duties.

Many articles were written by Dr. DeKay for professional journals. His research centered on tablet technology in the field of industrial pharmacy. He studied water soluble tablet lubricants, coating and disintegration of tablets, drying of tablet granulations by means of infrared and high frequency techniques and measurement of tableting pressure. In addition to the articles, he authored chapters in various undergraduate textbooks on the manufacturing of tablets. He also co-authored a book, *Qualitative Analysis for Students of Pharmacy and Medicine* and wrote *The DeKay Family in America* (1974).

In 1967, Mr. Ken Kiser, Purdue News Service, and J.V. Bothel wrote a tribute to Dr. DeKay in the "Indiana Pharmacist" (3: 93-94). Some of his attributes came through vividly in the prose. He was articulate, gregarious, modest and well-liked. We are also grateful for the memorial resolution written by Patrick F. Belcastro. We find that with all of the other activities, Dr. DeKay supervised all of the instruction and production of the pharmacy laboratory. This included liquid and solid medications for students dispensed in Purdue's pharmacy. He was also the X-ray technician and supervised a course for technicians. Biographies were found also in Wabash College's *Indiana Authors and their Books, Indiana Lives* by Hawkins and McClaren, *Indiana Scientists* by Visher, *Who's Who in Indiana*, and *American Men of Science*. Dr. DeKay was interested in photography, historical buildings and collecting pharmaceutical antiques. He traveled widely in pursuit of these hobbies.

It was the next year after Dr. DeKay came to Indiana, 1929, that he became a member of the Indiana Academy of Science. His interests were listed as Anthropology, Chemistry, Geology and Geography. A paper, "Objective tests in high school and general chemistry" was read by title in the Chemistry Division in 1937.

Dr. DeKay had many other affiliations with other societies such as: the American Pharmaceutical Association, Indiana Pharmaceutical Association, American Associa-

tion of University Professors, American Association of Colleges of Pharmacy, Rho Chi, Phi Lambda Upsilon, and Sigma Xi. He was National Secretary of Alpha Kappa Lambda (1946-1948) and National President (1948-1952). He served on the National Endowment Fund Board and was a member of the United States of America Pharmacopeia Revision Committee, Sub-committee 9 and 10 and collaborator for the *American Pharmacopeia* Volume 1 (1945) and Volume II (1947).

Dr. DeKay achieved much in his lifetime!

Herman Andrew Dettwiler

Monroe, Wisconsin — Greenfield, Indiana
March 1, 1910 — November 19, 1986

Dr. Herman Andrew Dettwiler was born in Monroe, Wisconsin, March 1, 1910, where he began his early education in kindergarten through second grade. The rest of grade school and high school were spent in Verona, Wisconsin, and college education at the University of Wisconsin where he earned a Bachelor of Science degree in 1935. He majored in bacteriology at Ohio State University receiving an M.S. degree in 1937 and Ph.D. degree in 1939. He was influenced probably in his choice of career by the Agricultural School professor in Bacteriology.

Dr. Dettwiler's professional career began in Cincinnati, Ohio, as Bacteriologist at Christ Hospital Institute of Medical Research, 1939-1940. He became a Bacteriologist at Eli Lilly and Company in 1940. He was associate director of the Biological Production Division from 1946 to 1952 when he was promoted to director. He retired in 1972.

When the first poliomyelitis vaccines were manufactured for public use, Dr. Dettwiler was director of its production at Lilly's. He was also interested in research on other viruses—especially influenza and typhus and in the production of influenza vaccine.

Besides his work at Lilly's, Dr. Dettwiler shouldered other heavy responsibilities, too. He was director of a Greenfield bank and Greenfield country club and was a member of the board of the Greenfield city schools from 1951-1957. He served on the Greenfield Chamber of Commerce, was a member of the Hancock County Bicentennial Committee (1976) and helped establish the American Revolution Bicentennial Memorial Park.

Dr. Dettwiler joined the Indiana Academy of Science in 1940 and was interested in the Bacteriology and Cell Biology Divisions. He served as Chairman of the Bacteriology Division in 1965. Other societies to which he belonged were: Sigma Xi, Alpha Gamma Rho, Alpha Zeta, New York Academy of Science, American Association for the Advancement of Science, American Society of Microbiology and American Society of Immunology. As hobbies, Dr. Dettwiler played golf and was adept at woodworking. He was a warm, friendly, hard-working man and believed that "what is worth doing is worth doing well". He died November 19, 1986, at his home in Greenfield, Indiana.

ROBERT HSI LIN HOWE

Swatow, China — Lafayette, Indiana
January 2, 1922 — July 29, 1987

Dr. Robert Hsi Lin Howe was a retired scientist, a professional engineer and consultant diplomat. Born in Swatow, China, January 2, 1922, and reared in that country, he came to the United States of America in 1948 and was naturalized in 1962. "The Lilly News" (Vol. 6, #25, December 8, 1962) carried a fine article about him at that time. He held five college degrees and spoke three languages. The degrees were as follows: B.S. degree in civil engineering from Methodist University, Soochow, China, 1943, and St. John's University, Shanghai, 1945; M.S. degree from Cornell University, 1949; Mechanical Engineering degree, Cornell University, 1950; and a Ph.D. degree from Purdue University in 1955.

Dr. Howe was a research and teaching assistant at Methodist University from 1943 to 1945 and instructor from 1945 to 1947. He became a research fellow at Purdue University, Lafayette, Indiana, in 1950-1952. In 1952, he became an engineer at Eli Lilly and Company and was soon a research scientist. He was in charge of waste treatment at the Tippecanoe Laboratories and consultant at other Lilly facilties. He retired December 31, 1982.

Recognition of his many talents came early in his career. His doctoral thesis on the application of aerial photographic engineering to water locating was basically the material in a movie widely circulated in other countries to help in solving problems of reclaiming land, finding more water and developing hydroelectric plants quickly and economically. His method of using aerial photographs to survey the land combined with his expertise about topography, soils, climate, geology, and vegtation resulted in locating water in a quick and easy way. He contributed many articles to professional journals and wrote *Applied Chemistry for Water Purification and Waste Treatment* I and II, 1970, and also *Handling of Hazardous Chemicals* I, II, and III, 1970 to 1973. Dr. Howe was a patentee, lecturer and science adviser abroad.

Dr. Howe received many awards: the Talbert Abrams Award given by the American Society of Photogrammetry in 1958, the gold award from the National Accountants Association, Industrial Wastes Award given by the Indiana Water Pollution Control Association, the William-Hatfield Award from the Water Pollution Control Federation, the Buswell Porges Award from the Institute of Advanced Sanitation Research, 1972.

Dr. Howe joined the Indiana Acadmey of Science in 1964 and was a family member. Mrs. Howe is a research chemist interested especially in food technology and biochemistry.

Dr. Howe gave many papers at the Indiana Academy of Science meetings and was a Chairman of the Environmental Quality Division in 1983. He gave as many as six talks at a single meeting a couple of times and he gave several at a single meeting other times. His diversity and the complexity of his work were shown in 1972 when he delivered six papers at six different Divisional meetings: Botany and Plant Taxonmoy, Ecology, Physics, Microbiology and Molecular Biology, Engineering, and Soil Science. He addressed other Divisions at other times. His work was usually directed toward solving problems in water purification and sewage disposal often using new methods of dealing with those problems. His work also led to basic research in biological processes such as oxygen production of algae and an interpretation of the mechanism. Environmental problems were also studied such as ozone and hydrogen peroxide injuries to green plants. He was also interested in the disposal and handling of dangerous chemicals.

Dr. Howe was active in other professional societies, too, as: Diplomate, American Academy of Environmental Engineers; Fellow, Royal Society of Health; American Public Health Association; Advanced Sanitation Reserach International (director of Council of Fellows, U.S. area 1970-1975); National Professional Engineers (chapter vice-president

1959-1960); and the American Chemical Society. Besides the "Lilly News" article, there is a fine biographical sketch in *Who's Who in the Midwest* (1976-1977).

Dr. Robert H.L. Howe died July 29, 1987, in Home Hospital, Lafayette, Indiana, after about a two-month illness.

VIVIAN ANNABELLE JOHNSON

Portland, Oregon
July 1, 1912

Lafayette, Indiana
June 30, 1985

Dr. Vivian A. Johnson was an emeritus professor of the Physics Department at Purdue University. She was born in Portland, Oregon, July 1, 1912, and educated there receiving a B.A. from Reed College in Portland, Oregon, in 1932. She came to Purdue University in Indiana, then, where she was associated with the Physics Department beginning with her graduate work which resulted in an M.S. degree in 1934 and a Ph. D. degree in 1937. She was a graduate assistant from 1932 to 1937, assistant instructor from 1937 to 1938, instructor 1938 to 1944, assistant professor 1944 to 1947, associate professor from 1947 to 1956 and professor from 1956 to 1979 when she retired and became an emeritus professor. She had been assistant head of the Physics Department since 1973. Dr. Johnson spent most of her time teaching undergraduate students who were non-physics majors, so she developed a course designed for the practical application of physics in other fields. She strove to modernize her courses and challenge students with higher standards.

In her research, the chief interest was theoretical analysis of the electrical properties of semiconductors. She presented over 30 papers at the American Physical Society meetings and contributed to the "American Physical Review", journals in Great Britain and the Netherlands. With Professor Lark-Horovitz, she edited *Methods of Experimental Physics*, and wrote chapters for other physics books. She prepared a biography of Dr. Lark-Horovitz for *Men of Physics* (1970). She also authored or co-authored with Dr. Lark-Horovitz some papers given at Indiana Academy of Science meetings. These concerned germanium, tellurium, and the Hall effect at very low temperatures. The semi-conducting properties of germanium were examined by a team of about 20 Purdue physicists, including Dr. Johnson, as part of the war effort. She joined the research team in 1942. We are indebted to the news releases and memorial resolution about Dr. Johnson sent from Purdue by the library archivist, Helen Q. Shroyer. The fine memorial resolution was written by Robert Lee Mieher and John W. MacKay. One item from the Purdue University News Service published in the "Purdue Alumnus" (Volume 61, #4, January, 1974) announced her appointment as associate head of the Physics Department and also mentioned that in 1973 she had received the Standard Oil Foundation Award for outstanding undergraduate science teaching. The memorial resolution also mentions that she received the Helen B. Schleman Gold Medallion Award in 1979 for her scholarship, leadership and service. Several other references were helpful as *Who's Who in Indiana, Who's Who in the Midwest, Indiana Scientists*, a Lafayette newspaper article, etc.

Dr. Johnson joined the Indiana Academy of Science in 1949 and had become an Emeritus member. As mentioned earlier, she gave several papers at Academy meetings. She was also affiliated with the American Physical Society, American Association of Physics Teachers, American Association for the Advancement of Science, American Association of University Professors of which she was Secretary-treasurer from 1945 to 1948, Sigma Xi (secretary of Purdue Chapter from 1941 to 1943), Sigma Pi Sigma, Alpha Epsilon Delta, Phi Beta Kappa.

Dr. Vivian Annabelle Johnson was 72 years old when she died at Saint Elizabeth Hospital, June 30, 1985, Lafayette, Indiana.

Ralph L(ouis Edwin) Seifert

Mount Vernon, Indiana
February 4, 1914

Bloomington, Indiana
April 23, 1987

Dr. Ralph L. Seifert was born in Mount Vernon, Indiana, February 4, 1914. His early education was obtained in southern Indiana graduating from Central High School in Evansville where he was a valedictorian. A Bachelor of Arts degree was received from Evansville College in 1934, and A.M. degree from the University of Illinois in 1935 and a Ph.D. degree also from the University of Illinois in 1937.

Dr. Seifert began his teaching career as an instructor in physical chemistry at the University of Illinois from 1937 to 1938. From 1938 to 1940, he taught chemistry at Alma College as an instructor. He was associate professor from 1940 to 1941, professor and head of the Department of Chemistry from 1941 to 1944. He joined a developmental program in chemistry at the University of Chicago Metallurgical Laboratory to support the war effort during World War II from 1944 to 1946. He was then an associate professor at Charleton College from 1946 to 1949 and head of the Chemistry Department from 1948 to 1949. He then joined the chemistry faculty at Indiana University in 1949 as an associate professor. He was made full professor in 1966 and retired as professor emeritus in 1978. He was co-author of the book *Physical Chemistry* (1966) with Dr. Frank T. Gucker. His research was focused primarily on the electrochemical study of molten salts and new techniques. He was on the advisory board of the "Journal of Physical Chemistry."

Dr. Seifert joined the Indiana Academy of Science in 1949 and was an Emeritus member at death. He was associated with the Chemistry Division. He was also a member of Alpha Chi Sigma, American Association for the Advancement of Science, American Chemical Society, Electrochemical Society, Sigma Xi and Phi Zeta. He is listed in *Who's Who in Indiana, Indiana Authors and their Books* (1917-1966) and *Indiana Scientists*.

Dr. Ralph L. Seifert died after a full and fruitful career April 23, 1987, at the Meadwood Health Center, Bloomington, Indiana. He had been ill for a long time and was incapacitated for the last few years.

IVA MIRIAM SPANGLER

Adams County near Decatur, Indiana — Fort Wayne, Indiana
February 14, 1898 — May 18, 1986

Miss Iva Miriam Spangler was an Adams County, Indiana, native and was educated through high school in Decatur. She was born at the end of the 19th century, February 14, 1898, and was 88 years old at death.

Miss Spangler first attended college at Ball State Teacher's College where she received a Bachelor of Science degree in 1927. In 1934, she was the recipient of a Master of Philosophy degree from the University of Wisconsin and in 1943 the Master of Arts degree from the University of Michigan.

Iva began teaching in Decatur and Adams Counties, Indiana, schools from 1915 to 1926. Then she taught in junior high schools in Fort Wayne, Indiana, from 1926 to 1937. In 1937, she went to Central High School in Fort Wayne. She taught there until 1964 when she retired from Fort Wayne Community Schools. During this time, she also served as an Indiana State Park Naturalist at Pokagon and Spring Mill State Parks (1944-1962). Then she was a biological science teacher at Indiana-Purdue University in Fort Wayne from 1964 to 1967.

Iva Spangler was a very accommodating and friendly person and knew plants and animals well. She was an excellent Park Naturalist and undoubtedly was a distinguished teacher. In 1961, she published a book *Flowers of Pokagon* (William A. Didier and Sons, Inc., Fort Wayne, Indiana) with an approach which was novel at that time inasmuch as the pages were color coded to match the flower color of the plant.

Miss Spangler joined the Indiana Academy of Science in 1937 noting interests as Botany and Zoology. She was an Emeritus member at death. It was not long after joining the Academy before she became the first sponsor of the Central High School Fort Wayne Nature Club which became a member of the Junior Indiana Academy of Science in 1940. She served as sponsor until 1963.

After retirement, she took annual trips to Mexico and Arizona besides participating in the Fox Island (Fort Wayne County Park) Bird Counts.

Miss Spangler had lived a long and productive life when she died May 18, 1986, at Fort Wayne.

William Stewart

Indianapolis, Indiana
February 7, 1931

South Bend, Indiana
May 22, 1985

Mr. William Stewart was a science teacher and pharmaceutical representative when he died in his home at South Bend, Indiana, on May 22, 1985. He was a native of Indianapolis, Indiana, where he was born February 7, 1931. He was raised and educated there until his graduation from Howe High School. He took his advanced work at Indiana University, Bloomington, where he received a B.S. degree in 1957 and then at South Bend where a Master of Science degree was obtained in 1983.

Mr. Stewart began his career as a salesman for the Geigy Pharmaceutical Company. His teaching career was spent at Grissom Middle School in Mishawaka, Indiana, where he began teaching in 1977. His subjects were Biology and General Science for seventh, eighth and ninth grade students. He set up a School Science Fair each year and had students who won awards in the Regional Science Fairs. He also coached girl's volley ball and track teams which he enjoyed very much.

Mr. Stewart joined the Indiana Adacemy of Science in 1980 as a family member with his wife, Kathy. Their interests were listed as Physics, Botany and Secondary Science Teaching. He became the treasurer of the Northern Indiana Regional Science and Engineering Fair. He was also associated with the National Science Teachers Association and Hoosier Association of Science Teachers, Incorporated. He was a precinct committeeman for the Republican Party.

As hobbies, gardening and travel held Mr. Stewart's interest. He enjoyed people and especially his students. His philosophy has timeless appeal—make the best of each day.

WILLIAM ARNOLD WILLER

Wheeling, West Virginia — Sodus, Michigan
December 21, 1898 — October 25, 1985

Mr. William Arnold Willer was born in Wheeling, West Virginia, December 21, 1898, and was a veteran of World War I. He started teaching early in life at 23 years of age in Howard County, Indiana, where he taught Science and English from 1921 to 1926. In 1927, he was teaching in Posey County. From 1927 to 1931, he was at New Albany. He moved to Fort Wayne in 1931 where he taught at North Side and South Side High Schools and Franklin Junior High School. He retired from the Fort Wayne Community Schools in 1965. His activities and subjects taught were a reflection of his continuing education. He received a B.S. degree from Purdue in 1927 where he was interested in Social Studies; so when he went to Fort Wayne, he taught Social Studies primarily. At the University of Louisville where he was enrolled in the Graduate School in 1929 and then again in 1946, his major was history and minor was education. His M.A. degree was conferred in 1946 and his thesis was "Origins of Secondary Education with Special Reference to Fort Wayne, Indiana". He received three certificates for teaching: High School Principal and Superintendent in 1937, registered high school teacher in 1932 (in Biology, Botany, General Science, Junior English, Agriculture, Chemistry, Physics and Physiology).

Mr. Willer joined the Indiana Academy of Science in 1929 designating Botany and Zoology as interests. He was the first sponsor for the Fort Wayne Junior Academy of Science Club, Franklin High School in 1945. He also was involved with the Fort Wayne Academy of Science. A small gift was given to the Indiana Academy of Science from the Fort Wayne Academy of Science by Mr. Willer in 1970 upon disbandment of the Fort Wayne Academy.

Mr. William Arnold Willer died at the home of his son, Paul F. Willer, with whom he was living. He was 86 years old at death on October 25, 1985, in Sodus, Michigan.

INDIANA ACADEMY OF SCIENCE
New Members for 1987

ABBOTT, JANE M., St. Mary-of-the-Woods College, Terre Haute, IN 47876
ADETOLA, OTUBU, P.O. Box 691586, Tulsa, OK 74169
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BANDY, JR., WILLIAM F., MSC-26, Vincennes University, Vincennes, IN 47591
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BERGDALL, KRISTIN, Physiology Department, Ball State University, Muncie, IN 47306
BERTA, SUSAN M., Dept. of Geog. and Geol., Indiana State University, Terre Haute, IN 47809
BLANCO, MARITZA, St. Mary-of-the-Woods College, Terre Haute, IN 47876
BLOME, STACY L., 436 Anderson St., Greencastle, IN 46135
BOLTMAN, BRUCE, 3609 Sycamore Drive, Columbus, IN 47203
BRADY, VALERIE, 321 Gerig Hall, Taylor University, Upland, IN 46989
BREWSTER, CHRISTINE B., 334 Hollowood Drive, W. Lafayette, IN 47906
BROOKS, CLARENCE, Dept. of Physics and Astronomy, Ball State University, Muncie, IN 47306
BROWN, DENNIS R., 2906 S. Taft St., Indianapolis, IN 46241
BROWN, MARCELINE, Psychology Department, Indiana University, Bloomington, IN 47405
BROWN, MARK A., Department of Geology, Indiana University, Bloomington, IN 47405
BUECHLER, RAYMOND J., Dept. of Anthropology, Indiana State University, Terre Haute IN 47809
BUTLER, DARRELL L., Dept. of Psych. Sci., Ball State University, Muncie, IN 47306
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CARPENTER, SCOTT, 4201 Grantline Rd., New Albany, IN 47150
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COLLETTI, ADRIA, SCBME, University of Notre Dame, Notre Dame, IN 46556
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HANSON, BRYAN A., Department of Chemistry, DePauw University, Greencastle, IN 46135

HEDGE, ROGER, Dept. of Natural Resources, 605 State Office Bldg., Indianapolis, IN 46204

HENDRICKSON, DONALD A., Dept. of Biology, Ball State University, Muncie IN 47306

HERENDEEN, PATRICK, Dept. of Biology, Indiana University, Bloomington, IN 47405

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HULVERSHORN, TOM, N. Central H.S., 1801 E. 86th Street, Indianapolis, IN 46240

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Part II
Addresses and Invited Papers

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PRESIDENTIAL ADDRESS

Biosensors: Recent Developments and Concerns

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Recent developments in biosensor technology have been hailed as the "marriage of the century—the marriage of biotechnology and microelectronics" (5). These developments, along with other advances in the field, have stimulated expectations that significant capabilities for monitoring and controlling an increasing number of biological and chemical species will be commercially available in the near future. My purpose in this talk will be to very briefly mention some of the important developments of biosensor technology which have occurred in the last twenty years and then focus on some of the very recent developments and possible future directions for this important emerging technology.

In order to appreciate the capabilities offered by some of the most recent advances, we need first to discuss some of the general classes and operation of biosensors. Biosensors can be classified in two general categories, devices which sense biological molecules and devices which use biological molecules as part of the sensing mechanism.

Biosensor technology is an outgrowth of ion selective electrode technology. One of the first and most commonly used ion selective electrodes is the glass electrode used for pH measurements. The first ion selective electrodes were very similar in size and use to the glass electrode. Similarly, the first biosensors were similar in size and use to ion selective electrodes—except that they either used or sensed biological or organic molecules rather than inorganic ionic species. For purposes of our discussion, I will categorize these biosensors as representing a "conventional" approach in contrast to some of the more recent devices which employ solid state microcircuits and/or fiber optics and which I will classify as the "microtechnology" approach.

Biosensors can be grouped into six major kinds:

1. Enzyme sensors
2. Microorganism sensors
3. Hybrid sensors
4. CHEMFETS/ISFETS
5. Multisensors
6. Optrodes

The first three of these are in the conventional category and the last three are in the microtechnology category.

Figure 1 shows a conventional biosensor in generalized form. The supporting membranes are normally porous or selectively permeable. The bioactive substance sandwiched between the supporting membranes can take any one of a number of different forms. Typical forms include an enzyme immobilized on a polymer substrate, a specific chemcial compound, a microorganism immobilized on an appropriate substrate or even a thin slice of plant or animal tissue which contains the desired enzyme or microorganism. The specific bioactive substance determines the type of conventional biosensor. Frequently, the biosensor contains a conventional gas sensing electrode above the upper supporting membrane.

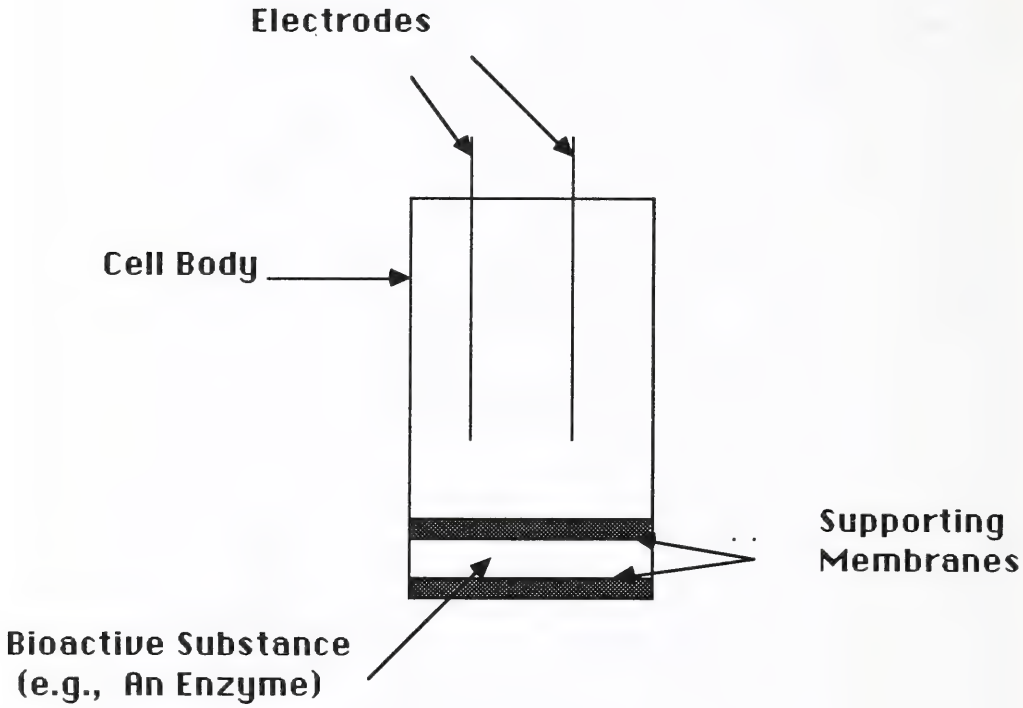

FIGURE 1. Simplified diagram of a generalized biosensor.

The general operation of a sensor consists of a chemical reaction catalyzed by or promoted by the bioactive substance. Typically, this reaction would either produce or consume a gas. The molecules of gas diffuse across the upper supporting membrane, which is selectively permeable to them, and cause changes in current or voltage at the electrodes. Monitoring these changes provides an indication of the amount of analyte present in the solution. Response times vary greatly with the specific electrode but generally are in the range of from 30 seconds to several minutes.

Since the enzymes or microorganisms are highly specific for a given type of reaction, the electrode is highly selective for a given type of analyte. The analyte molecules can be inorganic, organic or biochemical. Continuous or remote monitoring of concentration changes is possible. Many of the analytes for which biosensors are now available are species for which more traditional methods of anlysis are very tedious, time consuming and/or not easily adaptable for continuous or remote monitoring.

Applications for biosensors can be found in many diverse areas. Both measurement and control functions in environmental, physiological, agricultural, industrial and geological settings are but a few of the wide variety of areas which can or do already use biosensors. Advances in the microtechnology approaches permit extremely small sensors to be constructed so that remote, continuous, in vivo monitoring for several species simulataneously becomes possible in plants, animals and humans. Such techniques promise

to open the door to information about these systems which has previously been impossible to obtain and they offer the potential for greatly increasing our understanding of these systems.

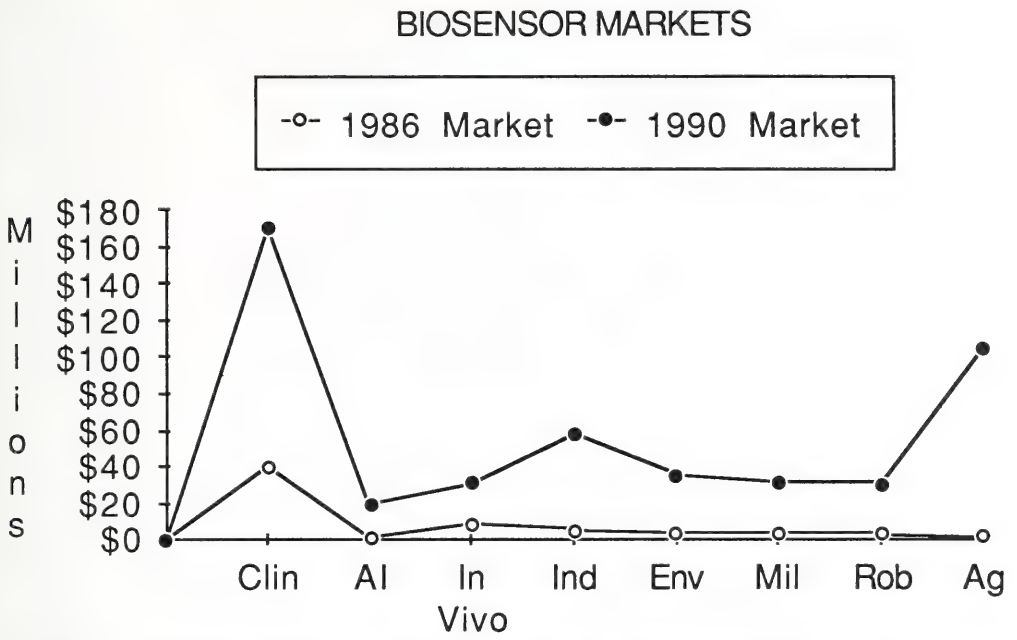

FIGURE 2. Projected markets for biosensors in various application areas. Clin = Clinical, AI = Artificial Intelligence, In Vivo = In vivo monitoring, Ind = Industrial application, Env = Environmental monitoring, Mil = Military, Rob = Robotics, Ag = Agricultural.

Figure 2 shows a plot of market research data for selected applications of biosensors based on data reported in 1985 (2). The lower of the two lines represents the market for biosensors in various areas in 1986 and the upper line the projected markets in 1990. Each of the application areas show is discussed briefly below.

Clinical

This area refers particularly to the development of multiparameter satellite medical testing facilities which can be installed in physicians' offices or taken to the bedside of hospitalized or homebound patients. This area shows the greatest market potential for the immediate future.

Artificial Intelligence

Biosensors can be used for feedback input into AI software for decisions regarding control and human intervention in chemical, physiological, enviromental or other types of processes.

In Vivo

Continuous and remote measurements in human, plant and animal systems are becoming possible using microtechnology and invasive techniques.

Industrial

Monitoring and control of bioreactors, fermentation broths, food production, etc. are areas offering great potential use.

Environmental

Hazardous substances in wastewater, air, etc., can be monitored continuously and remotely.

Military

Monitoring for toxic chemicals and biological weapons is a primary concern of military applications.

Robotics

Sensors to provide robots with touch, taste and tactile sensing will involve biosensors in many applications.

Agriculture

Quality control in food factories or monitoring soils for certain bacteria types are significant application areas. For example, a nitrogen fixing bacteria has been found to be the limiting factor for growing tomatoes. A bacteria has been developed which, when injected into the soil, increases tomato production. Monitoring these and other bacteria is necessary for continued use, research and application to other plants. This area is expected to offer a very substantial commercial market for biosensors.

CHEMFETS/ISFETS

Some of the most exciting recent developments in biosensor technology have been made possible by innovative applications of soild state microcircuitry and fiber optics. We will first consider the solid state sensors.

"CHEMFET" is an acronym for "chemically sensitive field effect transistor." "ISFET" ("ion sensitive field effect transistor") is another acronym for the same type of device. To appreciate this technology, it is helpful to review some characteristics of an insulated gate field effect transistor (IGFET).

IGFETs are tiny electronic devices which have for a number of years been used for amplifying or switching electronic signals. They are suitable for use in "keyless" control panels on microwave ovens, in elevators and cash registers. They can be manufactured to occupy a very small space, lend themselves well to integrated circuit manufacturing techniques and are frequently a basic component of solid state memories for computers. Literally thousands of these devices can be packed into a chip which is only one or two millimeters on a side.

Each IGFET typically has three leads designated as the source, the drain and the gate lead. If a current source, such as a battery, is connected between two of the leads (the source and the drain), a very small charge applied to the gate lead causes a significant change in the current flowing between the source and the drain. In the "keyless" control panels referred to above, the electrostatic charge on the operator's finger would be sufficient to change the current between the source and the gate leads from completely "off" to completely "on." The general principle is that a very small change in charge applied to the gate causes a relatively large change in the source to drain current. This is amplification.

In recent years, innovative chemists and physicists realized that there is no reason that the charge applied to the gate must come from a person's finger or from a voltage source which is part of an electronic circuit. Ions is solution would have the same effect. Therefore, if an IGFET were mounted on the end of a probe and modified so that when the probe was immersed in a solution, ions from the solution could come in contact with the gate, the current flowing between the source and the drain leads would be changed according to the number of ions contacting the gate. This concept gave rise to the chemically sensitive field effect transistor (CHEMFET) or ion sensitive field effect transistor (ISFET).

To make the ISFET selective for specific ions, a membrane which is permeable to only a given type of ion can be used to cover the gate lead. Only those ions which can permeate the membrane reach the gate and therefore effect the current through the ISFET.

Since ISFETs are easily fabricated with integrated circuit technology and can be made to occupy a very small space, they have been mounted on a tiny chip at the end of a standard catheter and used for in vivo monitoring of physiological fluids in humans and animals. The signal leads from the chip can be run through the catheter to the electronic monitors at a patient's bedside. This confirguration provides continuous readout of the physiological variables being sensed. Since several sensors can be fabricated on a single IC chip, several species can be monitored simultaneously. Typical sensors available for monitoring physiologic fluids include potassium, calcium, ammonium and sodium. An in vivo potassium monitoring system has been reported in which the performance was sustained for several hours. After the sensor was removed from the patient, it continued to give accurate measurements on standards for several hours. (3).

In vivo monitoring of physiological fluids is particularly attractive for use with critical care patients. Physicians now know that frequent interval measurements provide the first clues to potential dysfunctions otherwise unsuspected at that time. Critical care patients are commonly intubated with several catheters which could be used to also contain sensors for continuous monitoring without additional invasive procedures on the patient.

Possible future uses of continuous in vivo monitoring include the direct interface of the sensors with a computer. Using appropriate artificial intelligence software systems, current data could be compared with data banks and provide interpretations of trends in terms of differntial diagnoses—including the reporting of relative probabilities for different courese of action. Based on these interpretations, alarms could be sounded when critical values were encountered and suggestions made to the attending physicians regarding possible therapeutic interventions.

While biosensors interfaced to a data base with an expert system to interpret trends sounds somewhat esoteric, the feasibility of the system depends most strongly on the biosensor. The ultimate use of such systems will likely come to rest on the availability of rugged, accurate, reliable and safe sensors.

Ex vivo use of biosensors for monitoring physiological fluids is also possible and avoids some of the problems associated with the in vivo sensors. Biosensor systems for ex vivo testing have the potential to greatly reduce costs for blood and urine analyses. Since hospitals and the medical profession in general are being forced to become increasingly sensitive to costs for medical care, a large market appears to exist for ex vivo systems.

Flow injection analysis (FIA) ISFET systems will be able to provide the capability for ex vivo bedside multivariable testing. With these systems, a nurse would withdraw a fraction of a milliliter of blood from a patient through a catheter and inject the sample into a near bedside FIA ISFET unit. This unit would automatically test for a variety of blood or urine components. Such testing would decentralize frequent and routine testing and thereby reduce costs as well as reduce delay time between sampling and sample reporting.

While a qualified technican would likely be needed to calibrate such instruments in a hospital setting, the bulk of the testing would be done by nurses. Thus, substantial savings would be realized compared to staffing a centralized laboratory.

Another market for ex vivo biosensor systems is projected to be in individual physician's offices. Again, both cost and time considerations are likely to encourage the development and use of such systems.

MULTISENSORS

The ability to measure a number of different analytes simultaneously is a chracteristic of a device now under consturction by Molecular Devices. A four by four inch silicon wafer has room for over 1,500 different test sites. LEDs at the back of the chip can be

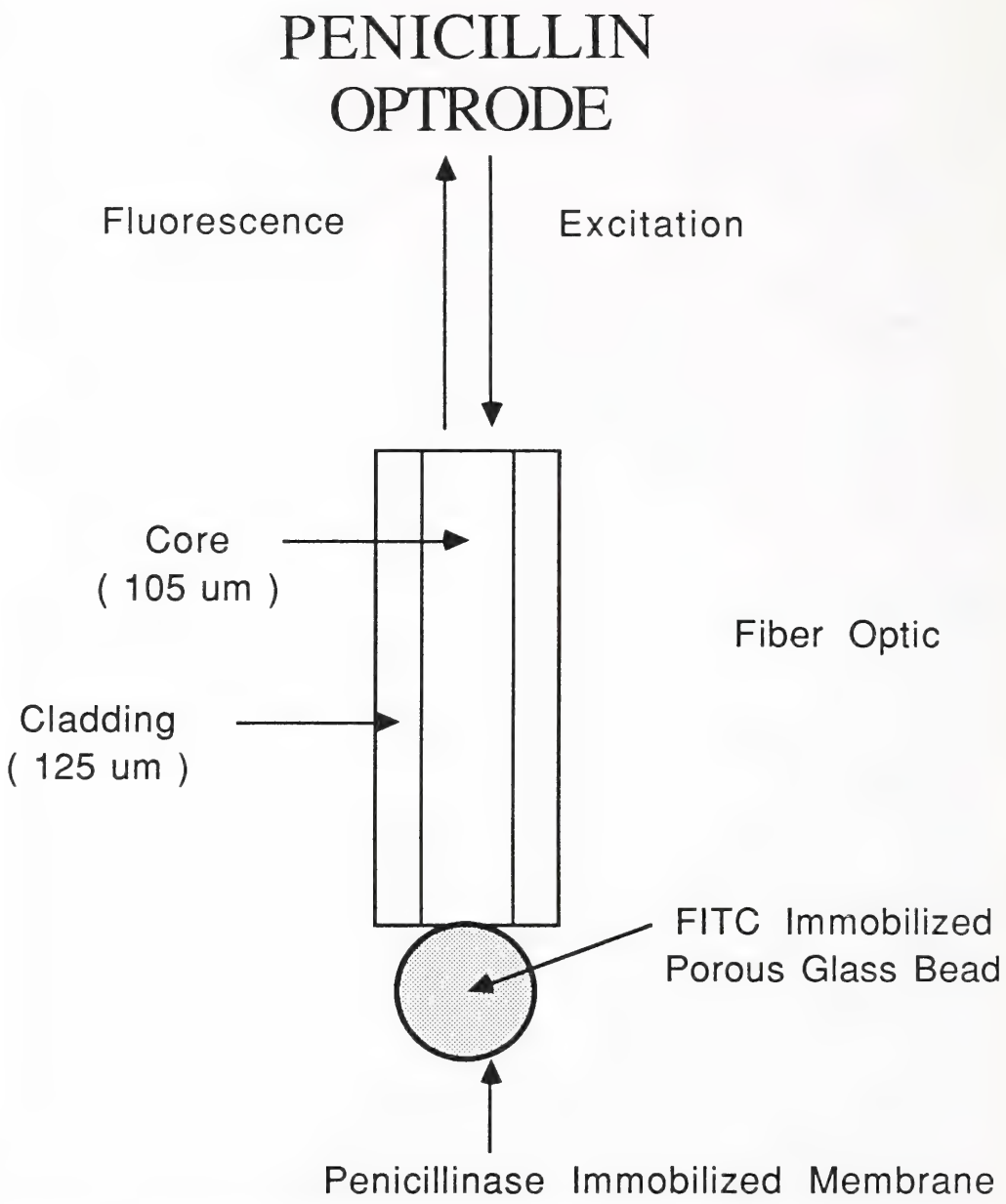

FIGURE 3. Penicillin optrode. Used with permission from Fuh, M.S., Burgess, L.W., and G.D. Christian. "Single Fiber Optic Fluorescence Enzyme Sensor." Preliminary manuscript for paper presented at FACSS meeting, November 1987.

turned on one at a time. The light emitted from an LED causes a photoresponse at a given area of the chip at which a specific test site is located. When there is a chemical reaction at that site, such as an antigen-antibody binding reaction in an immunoassay, the current flowing in that portion of the chip is changed and is detected. This device is scheduled to be marketed as early as sometime in 1988 (5).

OPTRODES

A characteristic of all of the solid state sensors listed previously is that they must transmit electrical signals from the sensor to the detector through electrical connections. This means that if they are used in environments in which magnetic fields exist or in situations in which stray currents can be hazardous (such as in in vivo situations) either

electrical interference or potential danger to the patient results. A relatively new approach which eliminates many of these problems involves the use of fiber optics to transmit the information from the sensor area to the dector. These devices are known as "optrodes."

Figure 3 shows a diagram of a penicillin sensor using this approach which was recently reported by Fuh, Burgass and Christian (1). This sensor consists of a small, porous, glass bead attched to the end of a single optical fiber. The bead contains fluoroscein isothiocyanate (FITC) immobilized on its surface. When radiation of an appropriate wavelength is transmitted through the fiber to the bead, the FITC fluoresces. The fluorescing radiation is transmitted back to the detector through the same fiber. The intensity of fluorescence has been shown to be proportional to the pH environment of the bead. Therefore, the fluorescence is an indication of pH.

The enzyme penicillinase can also be immobilized on the same bead. If the resulting bead is immersed in a solution containing penicillin, the penicillinase catalyzes the hydrolysis of penicillin to penicilloic acid and hydrogen ions. The increase of hydrogen ions at the bead surface causes changes in the fluorescence of the FITC on the bead. The resulting fluorescence has been shown to be linearly related to the amount of penicillin in the solution. The response time for this electrode is about 30 seconds and the working range in approximately 0 – 10 mM, although it varies somewhat with the buffer system used.

Another recent innovation employing fiber optics is the chemfuse (4). In contrast to the sensors described previously, these devices have only two significant output states rather than a varying output which is proportional to the concentration of some analyte. The operation of chemfuse is based on the fact that a change in some physical characteristics of a chemical (such as a change from the solid to liquid state) causes changes in its ability to reflect light. If a perturbation in the physical environment of this chemical occurs which is sufficient to cause the change in its physical state, the resulting change in reflective properties signals the presence of this perturbation.

Figure 4 shows the configuration of a generalized chemfuse. Suppose such a device were to be constructed so that it would indicate if a particular liquid chemical were spilled from a reactor. A solid substance which was soluble in the chemical of interest would be placed in the bottom of the 13-gauge hyperdermic needle. Light from the LED source would be transmitted through one of the optic fibers to the surface of the solid. A portion of the reflective light from the surface would be conducted to the detector through the second fiber. A steady state intensity of reflected light would result as long as the substance in the hypodermic needle remained solid. If the needle were subjected to an environment containing the spilled chemical of interest, this substance would enter the needle and dissolve the solid. Since the reflective properties of the resulting solution in the needle would be significantly different from the solid, a marked change in intensity of reflected light would be observed. This change could be used to sound alarms or initiate some automatic shutdown procedures. The solid substance originally placed in the needle could be chosen to be either very specific for one substance or specific for a range of substances.

Chemfuses are simple, small, rugged, and reliable. They require no calibration. As is characteristic for all optrodes, the light transmission is not effected by electrical or magnetic interferences which would cause problems for electriccal signals.

Chemfuses can be configured to act as temperature fuses by choosing the solid initially placed in the needle to have a melting point at the temperature of interest. A ferrographic sensor has been reported which detects the build-up of magnetic material in oil. This is of interest since small particles of iron are known to accumulate in, for example, motor oil, the longer it is used. The ferrographic optrode can continuously monitor the condition of the oil during operation of the motor and indicate the need for an oil and/or filter change. The ferrographic optrode is constructed by placing a small magnet, covered with aluminum foil, in the needle. Additional holes are made in the sides of the needle

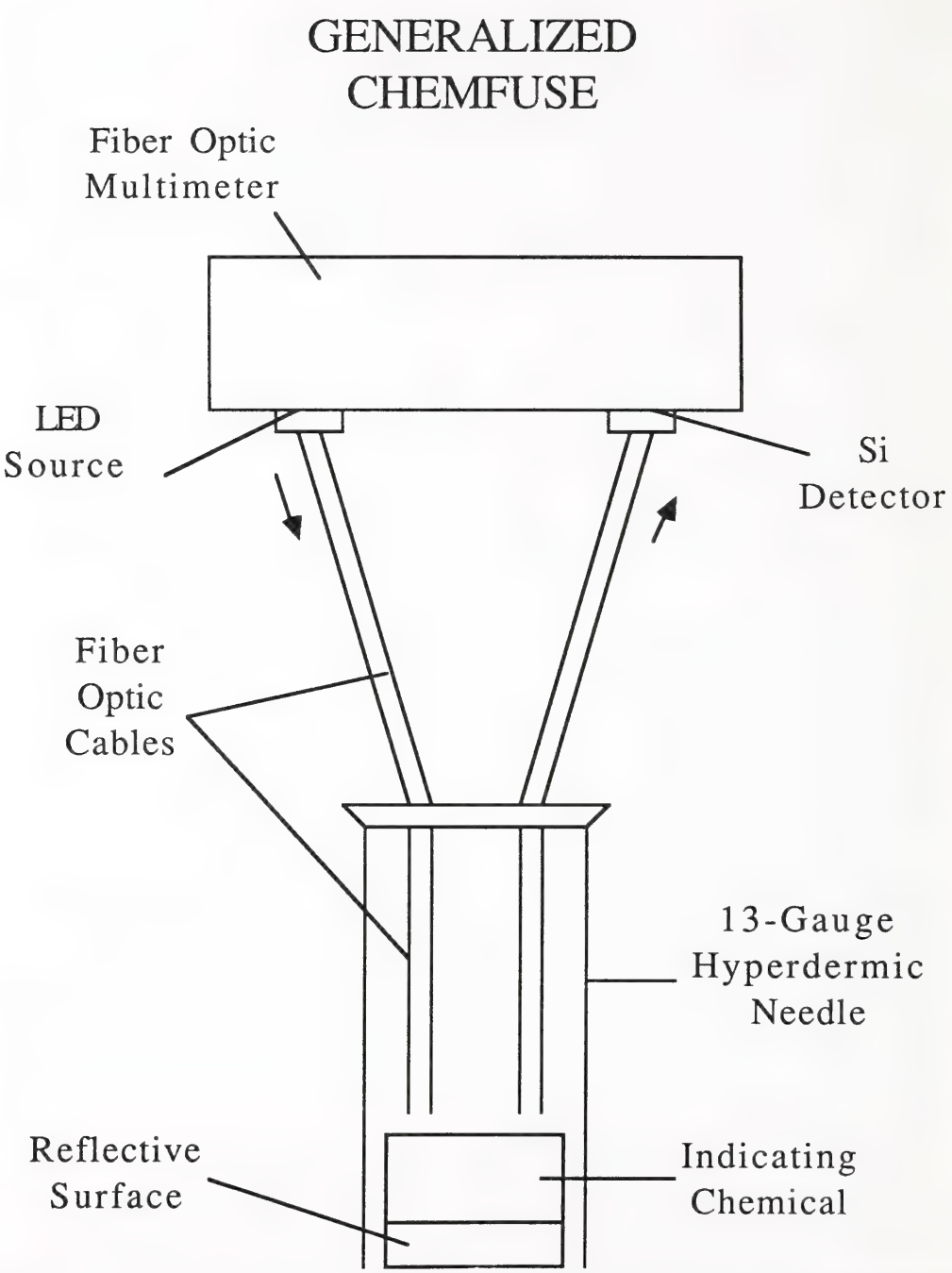

FIGURE 4. Generalized diagram of the chemfuse. Usd with permission and adapted from Tenge, B., Grunke, A.Q., and D.E. Honigs. "Chemfuses: Fiber Optic Switches Modulated by Chemical Substances." Preliminary manuscript for paper presented at FACSS meeting, November 1987.

and the needle is immersed in the circulating oil. Iron particles in the oil are attracted to the surface of the aluminum foil as they pass over it and adhere to the surface. The accumulation of these particles changes the reflective properties of the foil.

Since chemfuses are a very recent innovation, only a few possible uses have actually been reported. However, it seems likely that applications of the basic principles will be found for detecting many different substances, including many biological or biochemical substances.

CONCERNS

There are at least two primary areas of concern related to the development and marketing of biosensors. These are technical concerns and liability concerns. We will comment briefly on each of these.

In a Delphi study reported in 1985 (2), experts in the biosensor field were asked to rate critical issues relating to biosensor development. Problem areas were rated according to the degree of importance for the biosensor field to progress as well as their projected degree of difficulty in being solved. Figure 5 shows a summary of some of

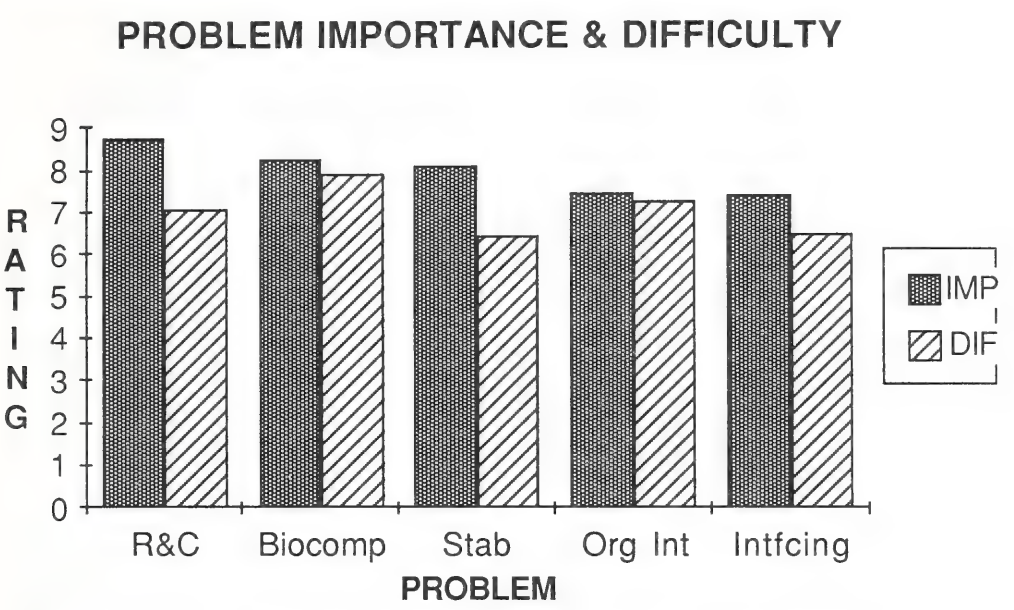

FIGURE 5. Five most important technical problems which need to be overcome for biosensor technology to progress rated by importance and degree of difficulty in solving.

these results. The five most important problems identified are shown and are: reliability and consistency of the sensor (R&C), biocompatability of the sensor with the environment/body (Biocomp), the stability of the sensor to changes in the environment (Stab), the sensitivity of the sensor to interferences from organic chemicals in the environment (Org Int), and the problems involved in interfacing with the biomaterial (Intfcing).

The left bar in each pair in Figure 5 is the rating of the degree of importance. The higher the number, the more important the problem. The right hand bar in each pair indicates the relative degree of projected difficulty in solving the problem.

Figure 5 shows that reliability and consistency (R&C) of the sensors is the most important problem by a slight margin. Interestingly, the solution to that problem is not seen as being as difficult as for some of the other problems.

The time projected for biosensor technology to penetrate several different market areas is shown in Figure 6. Of the areas listed in this figure, optrodes appear to have the potential to impact the market in the shortest time. Given the magnitude of market potential in dollars described earlier for clinical applications of biosensors, this might seem somewhat surprising. However, liability and ethical concerns are likely to delay the widespread use of biosensors for medical purposees in some areas.

Liability concerns are particularly severe in the medical field for in vivo applications of ISFETs. The potential harm to a patient resulting from a device failure or malfunction is likely to be a major impediment to the commercial introduction of these devices (3). Since there is a small electrical current flowing through the ISFET devices, if adequate

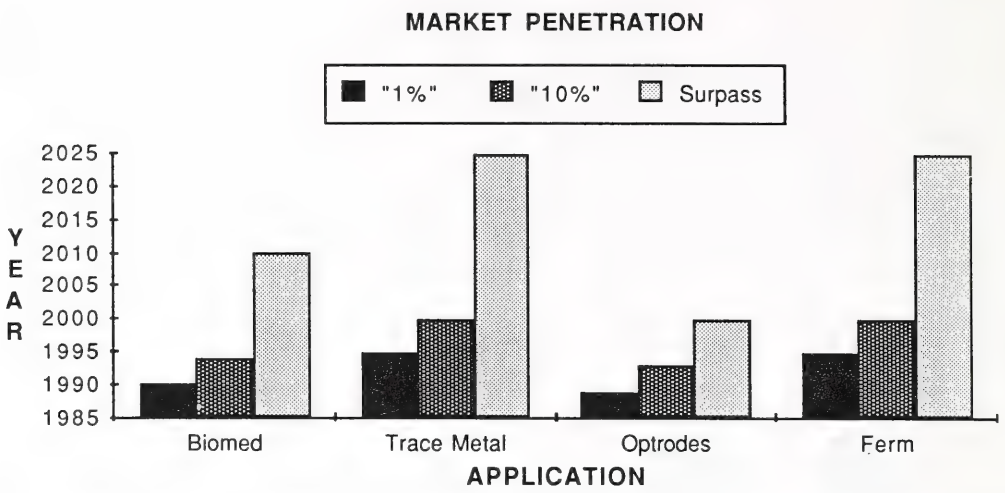

FIGURE 6. Time required for selected biosensor technology or application to penetrate the market place.

electrical insulation around the sensor were lost for some reason, potentially lethal damage to the patient would result. Stray current in in vivo systems could vere likely result in exogenous electrical stimulation of the heart and consequent myocardial fibrillation. In addition, as these devices are developed to the stage that they are incorporated into computerized AI systems used for differential diagnosis, automatic control or advice on therapeutic intervention, the risks rise substantially. Arranging for testing of these devices on human patients will likely be cumbersome and expensive at best and frought with the possibility of ethical considerations relating to the patient's comfort, safety and well-being.

While these considerations alone will likely not stop the ultimate development of in vivo applications of biosensors, they will very likely delay their introduction commercially on a wide scale. The fact that the Japanese have had some commercial versions of simple ISFET probes for in vivo pH and pCO_2 measurements available to the medical community since 1983 indicates that at least some manufacturers, medical personnel, regulatory agencies and patients are willing to accept the risks involved.

If U.S. companies are sufficiently reluctant to accept the liability risks, this reluctance will likely preferentially accelerate the development of ex vivo systems. In any case, biosensors appear to have a niche carved for them in a variety of applications and it is only a matter of time before widespread use of these devices occurs.

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"SPEAKER OF THE YEAR" ADDRESS—1987-88

The New Genetics: Applications and Implications For Our Future

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Introduction

In 1932, Aldous Huxley authored his famous look into the future entitled *Brave New World*. In this book Huxley predicted many things about the future of mankind and these predictions included the coming of man-made man: the test tube baby. Not only did he predict the coming of the test tube baby but he also predicted that it would take man 600 years to develop the technoloty necessary to create the test tube baby.

In Huxley's 1958 sequel entitled *Brave New World Revisited*, he reflected on his previous predictions. In the Foreword to *Revisited*, Huxley apologized to his readers for his failure to predict one event—perhaps the single greatest event—which had occurred during the preceding 26 years: the advent of atomic energy. However, he went on to make the following prophetic statement:

> The coming of atomic energy marks a great revolution in human history, but not (unless we blow ourselves to bits and so put an end to history) the greatest revolution. The revolutionary revolution will come not in the external world but in the souls and flesh of human beings.

Huxley went on to tell his readers to expect this revolutionary revolution in the world of biology. He also hinted at the rapidity with which this revolutionary revolution would come when he changed his original prediction regarding the timing of the test tube baby from 600 years to 100 years. Science and technology had progressed so far in one-quarter of a century that he was forced to modify his original prediction by five centuries!

If we accept the proposition that, included within Huxley's definition of "test tube baby" is any child which is the result of conception achieved under artificial circumstances in a laboratory environment, then we know that, had Huxley been correct in his 1932 prediction, he would have predicted the test tube baby not 600 years in the future, not 100 years in the future, but 40 years in the future! The first documented and substantiated reports of successful in-vitro fertilization were published in 1972—40 years after the publication of *Brave New World*.

As we approach the end of the 20th century, and gaze ahead into the 21st century, Huxley's prediction of a revolutionary revolution in the biological sciences and particularly in genetic engineering seems to be moving quickly and steadily toward reality. Some experts have observed and calculated that the information base in genetics alone is doubling every 18 months. This creates quite a dilemma because, although it is essential for us to be aware of these advances, it would be a full-time job to keep abreast by reading the scientific periodicals avalable to us. The purpose of this report is to give the reader a quick overview of a few of the exciting new advances in genetics.

Genetic Engineering

Genetic engineering refers to the area of genetics which deals with the shuffling of genetic material within and among organisms. This transfer of genetic material has

even been accomplished from animals to plants, an example of which is the human gene for Human Chorionic Gonadotropin (HCG) being transferred to and expressed in petunias. Now for the first time, we have plants which can produce HCG and can have positive pregnancy test results!

The concept of genetic engineering works because of one single fact: DNA is DNA is DNA. The DNA molecule which is contained in every cell of every organism is the same structure and has the same component parts. The DNA in human cells is the same as the DNA in bacterial cells which is the same as the DNA in grass and trees. Because DNA is DNA, parts of the DNA molecule from one organism can be spliced into the DNA molecule of another.

Recombinant DNA Technology

Recombinant DNA Technology refers specifically to the laboratory manipulation which makes genetic engineering possible. In order for the technology to work, it must be possible to cut DNA apart and paste it back together again. At the molecular level, however, this "cutting" cannot be accomplished with scissors or scalpel under a microscope but must be accomplished with enzymes in the test tube. The enzymes which cut the DNA are called endonucleases because they are able to cut the DNA within (endo) the molecule.

Enzymes are also used to "paste" DNA together. These enzymes are called ligases because they are responsible for the tying together or ligation of DNA parts.

Just as a surgeon must be able to manipulate his scalpel precisely in order to accomplish his task, so a geneticist must be able to cut DNA at specific points. Fortunately, each endonuclease recognizes and cuts only one specific sequence of nucleotide bases contained in the DNA molecules and this allows the geneticist the control necessary to accomplish his task. The "cut-points" in the DNA molecule are designated by the occurrence of palindromes. A palindrome is a sequence of letters, numbers, or nucleotides which reads the same from left to right as from right to left. Examples of palindromes include Hannah, radar, toot, and 100,001. Since DNA is a molecule which consists of a double strand of nucleotides and since these nucleotides always pair up the same way, the palindrome will "switch over" in the middle of the sequence. For instance, the GAA AAG palindrome will actually look like this on the double strand:

---- GAA TTC ----
---- CTT AAG ----

Since each endonuclease enzyme is restricted to a particular palindrome sequence, they are referred to as restriction endonucleases. The EcoR1 enzyme is the endonuclease which recognizes the nucleotide sequence used in the above example. It is interesting to note that the name EcoR1 refers to the enzymes' origin—it was isolated from the bacteria E.coli (Eco)—and the fact that it was the first restriction endonuclease so isolated (R1).

Application

The technology referred to above can be used to make multiple copies or clones of a gene. Many strains of bacteria contain circular molecules of DNA called plasmids. These circles of DNA can be isolated and manipulated in the laboratory. A DNA plasmid from E.coli can be cut open with one of the many restriction nucleases yielding an open circle. A human gene, for example, the gene for the protein insulin, can then be isolated from human white blood cells using the same enzyme. The human insulin gene will insert into the open circular plasmid from E.coli. The new larger circle of DNA is tied together with ligase and is called a recombinant plasmid because we have recombined human DNA with bacterial DNA. The recombinant plasmid can then be placed back into E.coli cells

by the process of transformation. As those cells grow in the laboratory, the human insulin gene will replicate every time the bacterial cell divides. These rapidly growing bacterial cells are actually making copies or clones of the human gene. The copies of the insulin gene can later be isolated from the recombinant plasmids by using the same restriction enzyme. When the E.coli plasmid is carrying the human gene, the plasmid is referred to as a replication vector.

If the human gene is inserted into a bacterial plasmid "downstream" from a promoter or on-off switch, the bacterial promoter can activate the human gene so it can be expressed. That means the bacterial cells which contain the recombinant plasmid can manufacture the human protein, i.e., insulin. The plasmid with the promoter used to express the gene is called an expression vector. This technology is now used by The Eli Lilly Company to produce an unlimited supply of pure insulin synthesized in the laboratory and marketed under the trade name Humulin. Previously insulin for diabetics had to be isolated from the pancreas of pigs and cows. This technology has also been applied to permit the manufacture of human interferon, growth hormone, Factor VIII, tissue plasminogen activator, tumor necroses factor, erythropoietin, and many others.

Some of these genes are too complex for E.coli to handle. For instance, the gene for Factor VIII which makes the protein necessary for the proper clotting of blood is made up of 186,000 nucleotide pairs and has 26 exons. The Factor VIII gene had to be inserted into Chinese hamster cells and was then expressed by the mammalian expression vector, in-vitro.

Another application of the technology is based upon analysis of DNA fragments. If DNA which has been isolated from an individual is mixed in a test tube with a particular restriction enzyme, the DNA will be cut into small fragments. The size of the fragments will depend upon the location of the cut-points or restriction sites on the DNA. Since the number and position of the restriction sites vary from person to person, the lengths of the fragments and the number of fragments will also vary. Once exposed to an endonuclease, an individual's DNA fragments can be separated using the process of electrophoresis. This process will result in an electrophoretic pattern which is unique to that individual.

It has been discovered that some specific electrophoretic patterns may be associated with a genetic disease. By using restriction fragment length polymorphisms (RFLPs) in some families it is possible to make presymptomatic diagnoses. For instance, Huntington's Disease (HD) is a slow, progressive, physical and mental deterioration of the central nervous system. It is caused by an autosomal dominant gene and; therefore, it is expected, by chance, to be passed from an affected parent to half of his children. Huntington's, however, may not manifest itself until the affected person is 35 years of age or older. In the past, the children of an individual with HD had to wait with great anxiety to see what would happen to them in mid-life. But today, in some families by using RFLPs, the geneticist can distinguish with great accuracy those young people who carry the HD gene. This can be done by evaluating several family members both normal and those affected with HD. If all of the normal individuals in the family have three fragments but those with HD have four, the extra fragment serves as the RFLP marker for diagnosis. RFLPs can now be used to diagnose numerous genetic conditions in certain families. These conditions include: Duchennes muscular dystrophy, polycystic kidney, cystic fibrosis, neurofibromatosis, and familial polyposis coli.

The new RFLP technology gives rise to many ethical questions: should the child of a parent with HD be tested for the HD gene? If so, when? Should RFLPs be used for prenatal diagnosis? Should an HD fetus be aborted knowing that person will be healthy for 35 years and knowing we may have a cure for HD by that time?

There are some RLFP differences that are so unique that no two individuals, except for identical twins, are the same. These are based upon the natural variation among

repeated DNA sequences within our non-functional (not protein producing) DNA. These fragment size differences are called VNTRs (variable number of tandem repects) and are being used in forensic science. The identification of a rapist can be confirmed by matching the DNA fragments from the suspect to the DNA fragments of the semen found at the crime site. It will be possible to identify a murderer by matching the suspect's DNA to hair, skin or blood left by the murderer at the murder site. In addition, another new procedure, polymerese chain reaction (PCR) permits very small amounts of DNA to be multiplied over a million-fold in a few hours resulting in the quantities of DNA necessary for RFLP analysis.

RFLPs are also the key to the human genome sequencing project. The goal of the project is to work out the entire human DNA sequence for all twenty-four (24) human nuclear chromosomes. The project is expected to take 10-12 years to complete at a cost of about 15 million dollars. The complete human sequence contains an estimated 3 billion nucleotide pairs.

Other discoveries and applications of the recombinant DNA technology can only be mentioned briefly: The discovery that some enzymes are RNA molecules called ribozymes instead of proteins has shattered the old "all enzymes are proteins" rule. Human mitochondrial DNA analysis has led to the realization that we all evolved from a common female ancestor now referred to as "Eve". DNA from an Egyptian mummy 2,400 years old has been isolated and cloned, as has DNA from the brain of a human found in a Florida peat bog that was 8,600 years old. Genetically engineered plants that are frost resistant have received the necessary legal approval for field testing. The T. plasmid, a tumor including plasmid from a bacterium, has been used to carry foreign genes into plants. The RFLP for muscular dystrophy has led to the discovery of the actual gene responsible for the disease and analysis of its product named dystrophin. This technology has also been instrumental in the discovery, analysis, and vaccine development for HIV, the AIDS virus.

As we look to the future, the applications of the genetic engineering technology appear to be infinite. Gene therapy via gene transplantation may soon be possible to correct some human genetic diseases. The therapy will involve obtaining bone marrow cells from the affected individual and isolating the pluripotent stem cells from the marrow. The stem cells will then be infected in a petri plate with a cancer causing virus which has been modified in two ways: the genes necessary for cancer induction have been removed and the gene missing in the affected individual has been inserted. The modified virus therefore serves as a vector to carry the "good" gene into the stem cells. Once the stem cells contain a new normal gene, they are put back into the patient. The new stem cells will give rise to new red and white blood cells which will carry the normal gene and make the missing protein. As the cells are distributed throughout the body they will also distribute the previously missing protein and, in so doing, correct the genetic defect.

Some investigators believe that placing genes into the fertilized egg might be a future possibility. It is already possible to inject the human gene for growth hormone into a fertilized mouse egg and get mice which are twice normal size. Other investigators have injected a cancer causing oncogene into mouse eggs and have produced mice which transmit the oncogene to their progeny. These mice have even been patented. As we look at the future implications of this work it is easy to speculate about using the same procedures to insert a gene into a human egg soon after conception to correct a human gene defect.

As progressive and prophetic as Aldous Huxley was, he did not have an inkling as to the tremendous and far-reaching possibilities of his revolutionary revolution. Today's scientific community has a two-fold responsibility as we look to the future. One. . . . to continue to innovate work in the laboratory and two. . . . to inform the lay public of the upcoming social implications of this work.

Future Implications of Genetic Engineering

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To understand the basis for future developments implicit in genetic engineering, it is applicable to review basic concepts and discoveries which support this area. Over the past decade, molecular genetic research has demonstrated that genes are able to function in almost any cellular environment and that this function persists even after substantial modification of gene sequences. Genetics and Biochemistry used to be considered as separate fields but in 1972, Jackson, Symonds and Berg at Stanford University fashioned a circlet of DNA containing a piece from a bacteriophage (bacterial virus) to which was chemically linked part of the DNA of a simian tumor virus also found as a circular gene (1). This work drew upon many decades of prior research conducted in the USA and internationally which commenced with isolation of the first bacterial viruses in 1915, a finding subsequently confirmed in many laboratories. By the 1960s, studies on the mechanism of various bacterial viruses had shown that certain types incorporate into the host genes after infection there to remain dormant for a variable period, while others multiply rapidly in a bacterial host, resulting in death of the host and release of virus progeny. This research also demonstrated the existence of circular genetic pieces in some bacteria which were extrachromosomal (outside the main body of DNA) and capable of autonomous duplication when a cell duplicated its main DNA. The circular pieces are called plasmids and the 1972 report from Berg's laboratory signified that a plasmid had been assembled which was of experimental rather than natural origin.

In the same year Cohen, Chang and Hsu also at Stanford, published a method for introducing circular plasmid DNA into the common laboratory bacillus *E. coli*, which exists in nature in the large intestine of humans (2). The following year, Cohen and Chang collaborating with Boyer and Helling at the University of California in San Francisco, announced that a plasmid carrying genes for resistance to antibiotics had been designed and inserted into *E. coli* where it was increased in quantity as the bacterium underwent its numerous cycles of doubling (3). This process of increasing copies of an engineered plasmid by inserting it into a fast-growing cell is one definition of the much-used term cloning. The early reports of recombinant-DNA technology, as this type of work is designated, include a demonstration that segments of complex cell DNA (eucaryotic cells), in this case from the African toad *Xenopus laevis*, could be enzymatically linked to plasmid genes of laboratory origin and cloned in simple cells (procaryotic cells) such as *E. coli* (4).

These pioneering discoveries are the foundations of a large body of research discoveries and biomedical applications utilizing gene construction or modification, followed by insertion of the novel gene into a cell where it multiplies (*replicates*) and its information may subsequently be *transcribed* into RNA, including stands of messenger RNA. Cells containing these synthetic genes can often be treated in a fashion which results in *translation* of the messenger RNA, resulting in a protein or peptide product. Readers who wish to review DNA replication, RNA synthesis (transcription) and protein synthesis (translation) will find a non-technical treatment in any college-level text of biology for non-majors. Figure 1 summarizes these relationships.

A list of hormones or regulatory molecules produced for medical or veterinary use by recombinant-DNA technology includes human insulin, human growth hormone (somatotropin), porcine growth hormone, interferon (a natural protein which inhibits viral invasion of animal cells) and somatostatin, involved in antagonizing the action of somatotropin. Many more peptide or protein factors are becoming available. These are

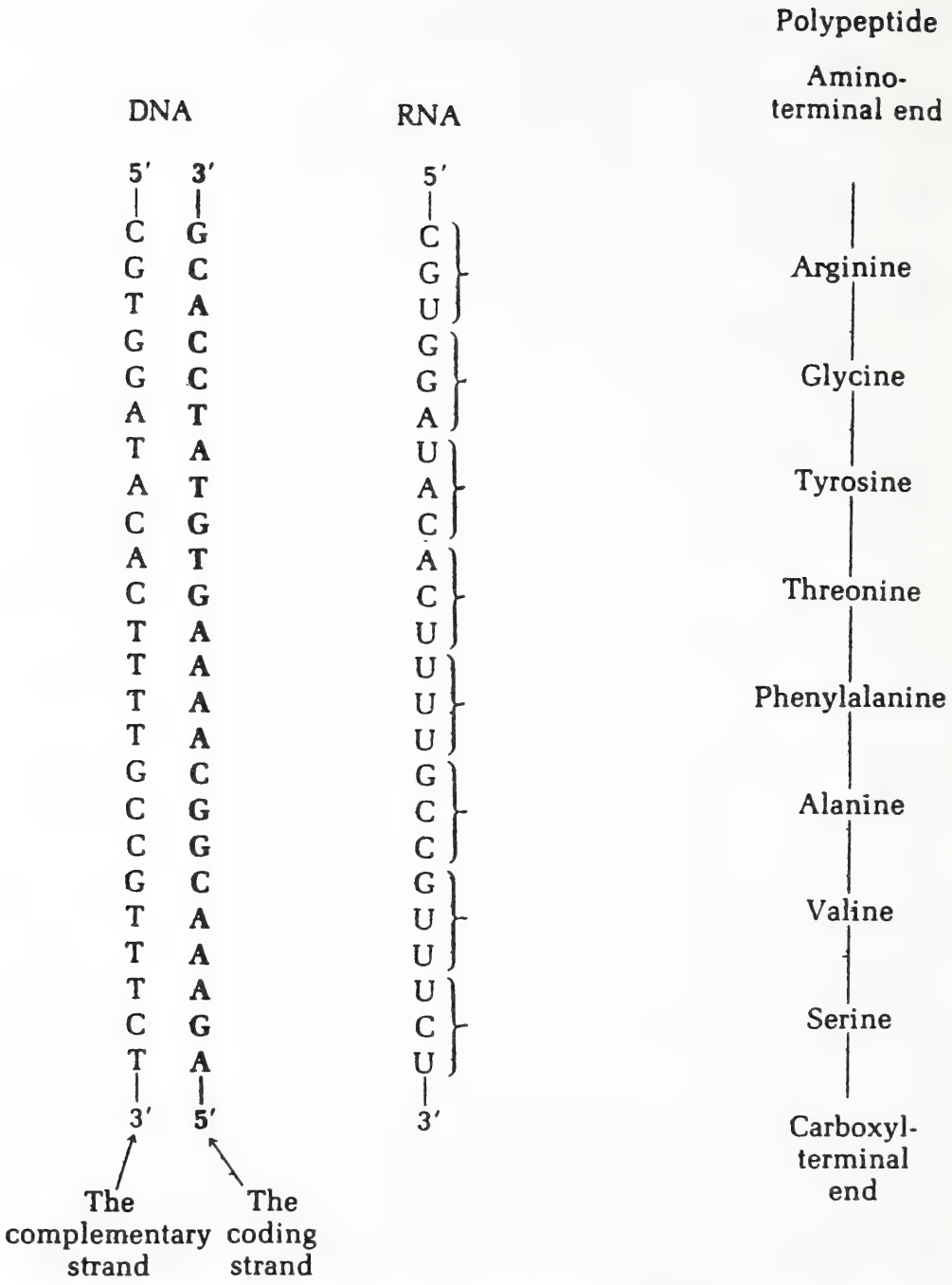

FIGURE 1. DNA Double Strand, Messenger RNA Transcript (complementary to Coding Strand) and translated Octapeptide. Triplets of nucleotides (bases) in DNA determine mRNA triplets which when translated determine the amino acid sequence in protein.

produced by introduction of enzymatically-modified plasmids into bacteria which subsequently multiply and synthesize the regulatory agent coded for by DNA in the appropriate plasmid.

In 1983, Brinster and collaborators at the School of Veterinary Medicine of The University of Pennsylvania described the incorporation of the gene for human growth hormone into the region of mouse DNA which codes for the synthesis of a regulatory protein stimulated by metals in the diet such as zinc. The synthetically produced fragment

containing DNA for human growth hormone, was then injected into the nucleus of a fertile mouse egg and the egg was implanted into the uterus of a laboratory mouse. When the mice were born they were fed a diet containing small amounts of zinc. Those which carried the synthetic DNA in their body cells responded by growing to a size larger than their litter mates which did not receive the human growth-hormone gene. One mouse from an egg which was injected with two copies of the recombined DNA grew to twice the size of its litter mates (5). Any animal altered by incorporating into its cells novel or alien genetic information is called a *transgenic* animal. The establishment of transgenic animals, where specific alien genes are incorporated into the germ line of fertile eggs, represents one of the most potent aspects of genetic engineering. This technology includes the possibility of establishing laboratory animal colonies carrying genetic anomalies identical to those which afflict humans. This is a bright prospect for future therapy for genetic diseases which comprise a large portion of the catalog of human suffering and tragedy. A comprehensive review of developments in this relatively new field has been published by Jaenisch at the Massachusetts Institute of Technology (6). Figure 2 illustrates the principal steps used for transgenic animal research.

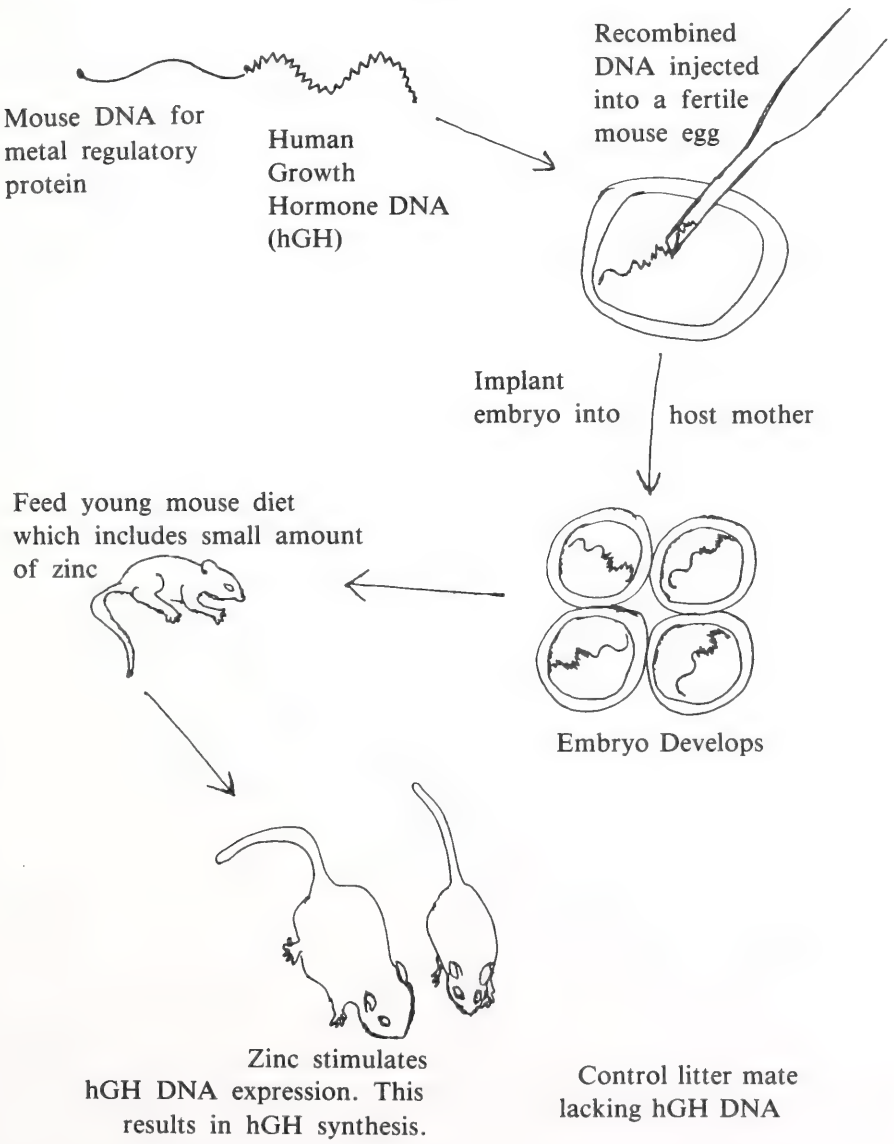
Mouse DNA for metal regulatory protein
Human Growth Hormone DNA (hGH)
Recombined DNA injected into a fertile mouse egg
Implant embryo into host mother
Feed young mouse diet which includes small amount of zinc
Embryo Develops
Zinc stimulates hGH DNA expression. This results in hGH synthesis.
Control litter mate lacking hGH DNA

FIGURE 2. Transgenic animal production.

The same enzymes used to make specific cuts in DNA of various species, along with other enzymes used to ligate or splice the DNA fragments together, can be utilized for the identification of marker regions of DNA near genes which code for specific observable characteristics in an individual organism. Readers familiar with genetics will recognize that all the observable characters in an individual comprise the *phenotype*. On a chemical basis, the phenotype of an individual consists of all the proteins synthesized during translation. Controlling the individual proteins are the individual sequences of DNA, each containing the four chemical nucleotides or bases A, C, G, and T in linear array. These sequences code for the linear sequences of amino acids in each protein. The linear amino acid sequences in the DNA are the *genotypes* or genotypic features. Figure 3 shows the nucleotide code and its amino acid equivalents. Another standard term is *trait* which is used for any variaiton of an inherited character or characteristic. Eye color is a trait. When referring to variations in eye color, each color is called an *allele*. Every allele of a trait represents a mutation in the DNA.

First letter	Second letter: U	Second letter: C	Second letter: A	Second letter: G	Third letter
U	UUU Phe	UCU Ser	UAU Tyr	UGU Cys	U
	UUC Phe	UCC Ser	UAC Tyr	UGC Cys	C
	UUA Leu	UCA Ser	UAA Stop	UGA Stop	A
	UUG Leu	UCG Ser	UAG Stop	UGG Trp	G
C	CUU Leu	CCU Pro	CAU His	CGU Arg	U
	CUC Leu	CCC Pro	CAC His	CGC Arg	C
	CUA Leu	CCA Pro	CAA Gln	CGA Arg	A
	CUG Leu	CCG Pro	CAG Gln	CGG Arg	G
A	AUU Ile	ACU Thr	AAU Asn	AGU Ser	U
	AUC Ile	ACC Thr	AAC Asn	AGC Ser	C
	AUA Ile	ACA Thr	AAA Lys	AGA Arg	A
	AUG Met	ACG Thr	AAG Lys	AGG Arg	G
G	GUU Val	CGU Ala	CAU Asp	GGU Gly	U
	GUC Val	GCC Ala	GAC Asp	GGC Gly	C
	GUA Val	GCA Ala	GAA Glu	GGA Gly	A
	GUG Val	GCG Ala	GAG Glu	GGG Gly	G

FIGURE 3. The Genetic Code in its RNA form. AUG also serves as a start codon. Three of the sixty-four triplet condons are stop codons. A few codons differ in the mitochondria. Otherwise the code appears to be universal.

In humans, the total collection of genetic information or *genome* contains between 50,000 and 200,000 genes, most of which either code for specific proteins or for RNA molecules which are either translated or play a role in translation (7). The thousands of genes are distributed over 46 pairs chromosomes and estimates are that there are some 3 billion bases (nucleotides) in double strands of complementary DNA, which make up the entire genome. Possibly only 10 percent of all the bases are ever expressed. Recent

work has revealed large sections of DNA present in repeat or redundant sequences and other sections contained in pseudogenes which are defective and can never be expressed (8). Over the past decade, several laboratories have concentrated on applying recombinant-DNA methods to the identification of markers associated with human genetic disease. Dr. Helen Donis-Keller of Collaborative Research in Bedford Massachusetts has reviewed progress in this area and states that, as of 1985, two hundred and forty-nine human genes had been assigned to specific chromosomes (9).

In many cases, the allele which calls attention to the existence of a specific gene is involved in a genetic disease, due to a mutation in the DNA which interferes with or prevents expression of the normal trait. By use of enzymes known as restriction endonucleases, since they make specific cuts in DNA without completely breaking it down into its four constituent bases, a number of enzyme-sensitive sites have been detected in human DNA (as well as that from other species). Many restriction endonucleases are now available from commerical suppliers and with these, a large number of non-overlapping restriction sites have been reported in human DNA. Dr. Donis-Keller points out that as of 1987, some 376 specific sites known as Restriction Fragment Length Polymorphisms or RFLPs have been assigned to specific human chromosomes (9). This means that enzymatically sensitive sites are mapped as RFLP loci and these provide the opportunity for cutting the huge pieces of chromosomal DNA down to more manageable fragments. It is important to emphasize that the RFLP loci or markers have no causal connection with the genetic diseases, but rather serve as signposts to aid in locating the disease-associated region for further study.

Figure 4 shows the main procedures used for RFLP identification. In the figure, white blood cells or other body cells are obtained from the parents, an affected child if there is one, and the fetus at risk for inheriting the condition or disease. Fetal cells are obtained either from aminotic fluid drawn at 16-18 weeks of gestation or chorionic villus samples obtained at 8 to 10 weeks of gestation. The cells are cultured, the genomic DNAs isolated and then each DNA isolate is treated with the same restriction endonucleases. The fragments of DNA are separated by size using an electric current passed through a sieve-like gel. After separation, the fragments are transferred to a nylon-like membrane to which they are made to bind tightly using specific environmental conditions. Then a radioactively labeled complementary DNA piece called a probe is incubated with the DNA fragments now bound to the membrane as single strands. The probe is from regions near the disease gene. After this incubation, the non-bound probe pieces are washed away and the gel is exposed to X-ray film. Within one to two weeks the film is developed and examined to see if RFLP fragments of the child at risk bind the probe made from regions associated with the disease. This method depends upon RFLPs which are spatially close enough to migrate (or during sex-cell production to cosegregate) with the gene that causes the disease. In figure 4, the fetus at risk has alleles 3 and 4 and is free of the disease. The mother and father (M and F on the autoradiograph) each carry the RFLP marking different alleles for the disease. In this illustration, the alleles numbered 1 and 2, if present individually, confer a carrier state on each parent. The markers are both present only when the child is homozygous (carries a marker from each parent). Accordingly, the fetus in question is not a carrier of either marker allele. Donis-Keller points out that a useful RFLP must be closely linked to the disease locus, where the criterion is not over 10 centimorgans away from the actual disease allele. (One centimorgan is 1 million nucleotides or base pairs). It is also important that the RFLPs flank the disease locus.

It has been estimated that sequence differences in the DNA of individuals occur on average, every 50 to 100 nucleotides. This permits large variation to exist when DNA is treated with a specific set of restriction endonucleases. Applications of this heterogeneity are now appearing in forensic analysis of blood, semen or tissue samples recovered where violent crimes have taken place. This application has already resulted in jury convictions

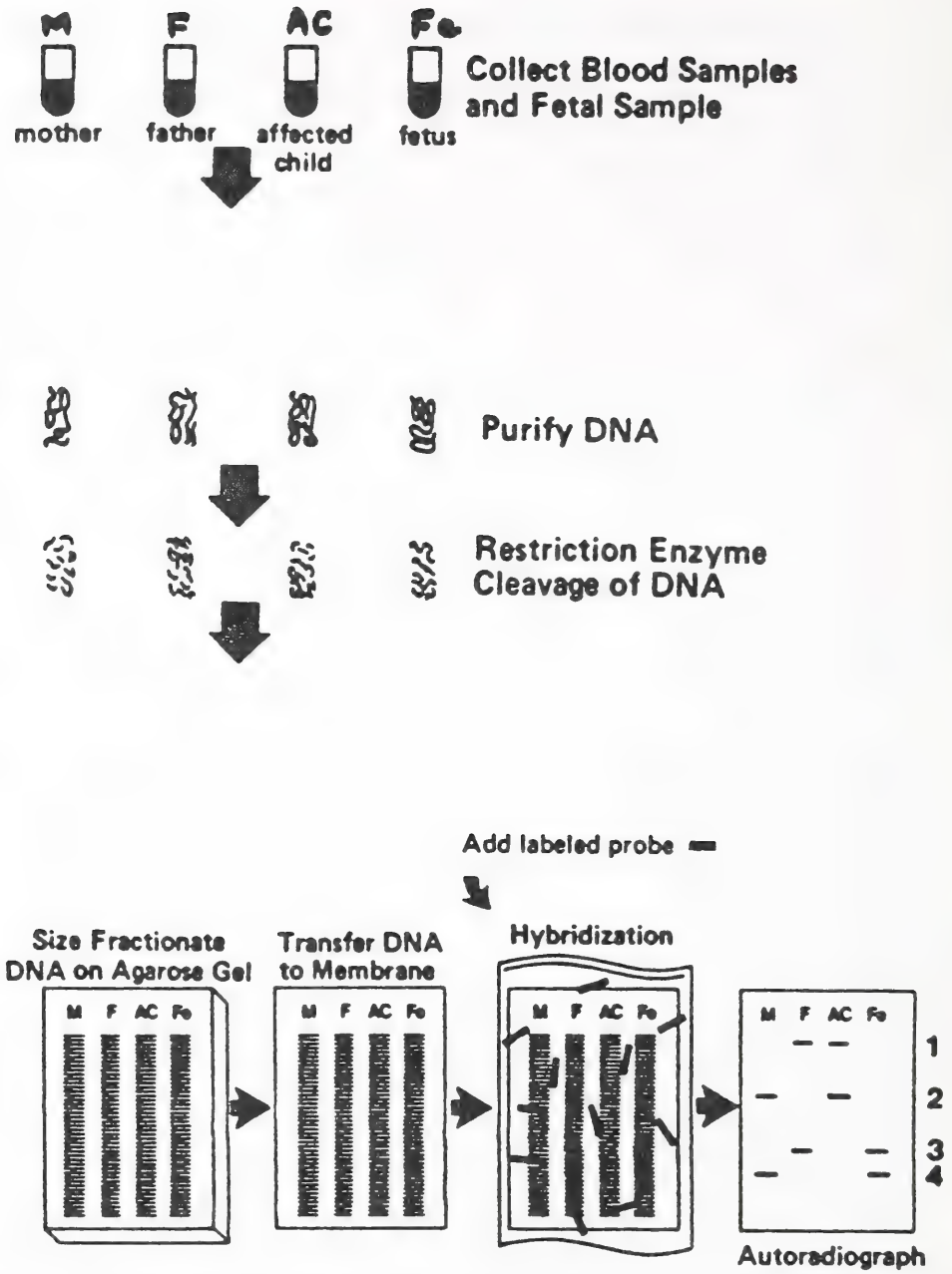

FIGURE 4. Restriction fragment length polymorphism (RFLP) identification steps.

of suspects in England and America. But the most future-directed application is the current work described by Donis-Keller where the goal is discovering genetic markers for various diseases and assigning these to specific chromosomes. A relatively complete map has been made of chromosomes 7, 16 and X. Partial maps exist for 12, 13 and 21. The first RFLP marker used for a probe was linked to the cystic fibrosis gene, at a distance of 15 centimorgans away, on chromosome 7. Since that report in 1985 (9), the list has increased and by 1987, RFLP probes were available for more than 40 inherited disorders. While this work is progressing, a lively debate has ensued over a proposal for federal support for mapping the human genome (10). This project would require an increase in funds and a commitment to a long-term program the ultimate goal being a complete sequence of the human genome. One of the liveliest proponents is Dr. Leroy Hood of the California

Institute of Technology. Recently Dr. Hood presented the argument to the medical research community (11). Hood has greatly improved machine methods for sequencing DNA and believes that the "proposition to map and sequence the human genome . . . is undoubtedly the most ambitious project ever conceived by biologists". Perhaps no development has more potential to bring about projects associated with this proposal than the increase in aged in America, Northern Europe, the Soviet Union and Japan. Currently, over 12 percent of all Americans are 65 or over, and by the year 2025 this number will have reached about 20 percent along with similar numbers in Northern Europe. At that time Sweden and West Germany will lead the world with 22 percent of their population aged 65 and over (12). The relationship to gene mapping and sequencing arises from the growing body of experimental work which shows genes in human DNA with the potential for causing transformation of cells to a cancerous state. Further, some biologists postulate that control of aging and the diseases of aging are all of genetic origin. Mapping and sequencing of the human genome, utilizing genetic engineering technology, offers a unique opportunity pertinent to these issues.

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Proceedings of the Indiana Academy of Science

Part III Highlights

DePauw's Dr. Welch Honored by N.Y. Botanical Garden Publication

The New York Botanical Garden has devoted a special issue of its scientific journal Brittonia to the work of Winona Welch, DePauw University professor emerita of botany and native of Jasper County, Indiana. The issue's date, May 5, was Dr. Welch's 92nd birthday.

The tribute recognizes the transfer last year of the Truman G. Yuncker Herbarium from DePauw to the garden.

Dr. James M. Hester, president of the New York Botanical garden, called the Yuncker Herbarium "the most significant acquisition of research specimens since the

1940s when the garden acquired the Princeton University collections." The garden has the largest herbarium in the U.S.

THE 135,000 SPECIMENS from the Yuncker collection represent the life research of Dr. Welch and Dr. Yuncker, who was her predecessor as chairman of Botany and Bacteriology at DePauw. He came to the faculty in 1919 and served as department head until 1956, then as curator of the herbarium (named by DePauw in his honor) until his death in 1964.

Winona Welch was his first major student, graduating in 1923. She returned to the DePauw faculty in 1930, becoming department head in 1956 and continuing herbarium work until her eyesight failed a few years ago. She had begun working with the herbarium in her student days and was its curator after Dr. Yuncker died in 1964. Together they represent more than a century of service to DePauw and of collecting for the herbarium.

Dr. Patricia K. Holmgren, director of the NYBG Herbarium, came to Greencastle to present the first copy of the commemorative issue of Brittonia to Dr. Welch personally. She was joined in the ceremony at Asbury Towers, where Dr. Welch lives, by DePauw President Robert G. Bottoms.

"IT IS RARE INDEED for research collections of the magnitude and classic quality of the Yuncker Herbarium to become available. The garden is pleased indeed to have the opportunity to make these valuable specimens available to researchers throughout the world." Holmgren said.

DePauw's decision to approve the garden's purchase of the Herbarium was made in deference to Dr. Welch's wishes.

Calling the 40,000 specimens of mosses and liverworts her "life's savings," Welch said she decided "it would serve science best to have all the collections at the New York Botanical Garden where they are accessible to botanists everywhere in the world."

Dr. Welch is the world authority on two families of mosses, the Fontinalaceae (water mosses) and the Hookeriaceae (a tropical family). Her monographs still serve as standard references, as does her pioneering volume, Mosses of Indiana.

Remarks made by Dr. Patricia K. Holmgren, Director of the Herbarium, The New York Botanical Garden, at the formal presentation of the Festschrift

Presentation of Winona H. Welch Festschrift, 5 May 1988

To know Winona Welch is to respect and love her. We are here today to pay tribute to this remarkable lady who combines boundless optimism with a talent for friendship. I have thought a long time about what I wanted to say on this day, but how can I who have known Winona for only a year, tell you, her lifelong friends, anything you don't already know about her. It finally occurred to me that I should simply read to you a few of the statements written by the many friends who have contributed to this volume in her honor. This sounds simple, but to select a few quotes when this entire volume is filled with quotable statements about her was not an easy task. This in itself reveals the genuine affection so many have for Winona.

Listen to what is said about Dr. Welch as a person.

> Dr. Winona Welch impresses all who meet her with her strength, her intellectual vitality, and her unaffected joy in life. It is not given to all of us to live so long; it is given to even fewer of us to have lived so well.
>
> Truly, she is a good person. I don't think she is capable of doing a mean or selfish thing. I can't believe that she has an enemy in the world.

Her brilliance, her energy, her perseverance, her insistence on quality, and her love of people are all attributes that make her life such a productive one.

She is the salt of the earth.

There is a trail of Winona's good deeds that won't be erased soon. Dr. Welch was a role model.

Listen to what is said about Dr. Welch as a teacher and researcher.
Dr. Welch's achievement as a scientist would be impressive in anyone. It is especially impressive when one remembers that through all these years, she was carrying a full teaching load. If in science she found her intellectual satisfaction, in teaching she found her purpose and direction. One suspects that the honor that touched her heart most deeply was to be considered one of the outstanding teachers at DePauw University. She was a born teacher, a natural teacher, efficient and conscientious but also enthusiastic, sympathetic, and beloved.

Gifted individuals, such as Drs. Yuncker and Welch, possessed a genius for recognizing talented students, arousing their interests, and kindling them with the motivation to tackle graduate school.

Winona Welch was a charmingly pleasant teacher and a loved and thoughtful friend ever attentive to the wishes of her students.

She had a dominating influence on the direction of my career. She opened for me a new world of beauty but also organization and logic.

She was the one person who always believed in me.

I am constantly reminded of the admiration and affection you have generated among former students. You are their mentor, their inspiration, and most important, you are their friend.

She was an excellent scientist—she has been a *great* teacher.

Winona was a phenomenon—a legend on the DePauw University campus—because of her stringent work habits.

Winona will be long remembered for the carefulness and the scholarly thoroughness of her work.

Admiration for this work was universal.

One of the absolutely best monographs ever written in the world in bryology.

Comments from her family.
You have been a loving and knowing sister, kind to everyone with whom you came in contact.

To the Lee children and grandchildren, she is beloved Auntie Winona as she was to us. Now that our parents are gone, when we "go home" it is Winona to whom we go.

Winona, Betty-Jane, and Barbara are still "family," still part of each other's lives, still sharing and enjoying memories.

Comments about her as a leader.
Dr. Welch has long been a pillar in our Academy's operations. In 1948,

she became its 63rd president, as the first woman to hold that office.

The high respect of your colleagues was symbolized in your selection as the first woman to head a science department.

She became the elder statesman of the "moss ladies," always a little ahead of the rest of us.

Without question she was the best secretary-treasurer the Society has ever had. Winona is by nature a well-organized, neat, and tidy person. She kept meticulous records and frugally guarded the treasury against any excesses and extravagances.

She is so exemplary a secretary of an organization that she has always been in great demand.

And now we know her as the inspirational leader at the retirement home.

In spite of three attempts she never did get the hang of retiring. At age 72, she is quoted as having said, "You'll just have to change your mind about what people this age can do." Meeting her today, you would have to change your mind about what people aged 92 can do. The gradual loss of her eyesight could have defeated an ordinary person. To her it has been a challenge. I am still amazed that she has read every word of this Festschrift herself on her visual aid machine, too modest to allow anyone else to read the many nice things being said about her. "Oh mercy," she exclaimed, when she learned that we actually planned to publish all these tributes, amazed that we should find extraordinary what she considers ordinary.

Charming, gracious, beautiful, Winona is above all visibly happy. Firm of voice, clear of mind, large of spirit, and active as all get out.

Well, Winona, I haven't mentioned the word herbarium once in this presentation, and that after all, is what brought us together in this very special friendship. You and Dr. Yuncker assembled one of the best small research herbaria in the country. Its value to the scientific community is enormous. There is no question that this is the most significant single acquisition of specimens by the Garden since 1945 when the Princeton University Herbarium came to the Garden. It is rare indeed for research collections of the magnitude and classic quality of the Yuncker Herbarium to become available. The Garden is pleased indeed to have the opportunity to make these valuable specimens available to researchers throughout the world. We are and I am personally, most grateful to you for your decision, which has given me the added pleasure of producing this Festschrift for such a deserving person. Those of us at the Garden who have come to know you have been delighted and enriched. It is an honor to present this tribute to you with our gratitude, with our admiration, and with our affection. Happy birthday, Winona!

Proceedings
of the
Indiana Academy
of Science

Part IV
Section Meetings and Contributed Papers

ANTHROPOLOGY

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ABSTRACTS

A Shoreline Reconnaissance of Cecil M. Harden Lake, Parke County, Indiana. C. Michael Anslinger, Indiana State University Anthropology Laboratory, Terre Haute, Indiana 47809.——During the late fall and early winter of 1986 members of the Indiana State University Anthropology Laboratory conducted a shoreline reconnaissance of Cecil M. Harden Lake in Putnam County. Of the 170 archaeological sites documented, few contained deposits with contextual integrity. Instead, most sites were found on severely deflated surfaces. Nevertheless, results of an analysis of lithic tools and waste debris collected from these sites provides a basis for assessing the nature of prehistoric settlement and resource exploitation within the study area.

Archaeological Excavations at the Middle-Late Archaic Bluegrass Site (12W162), Warrick County, Indiana. C. Michael Anslinger, Indiana State University Anthropology Laboratory, Terre Haute, Indiana 47809.——During the summer and fall of 1987 the Indiana State University Anthropology Laboratory conducted a "Data Recovery Phase" excavation at the Bluegrass Site (12W162), in Warrick County. Previous test excavations were made at the site by the Indiana State University Anthropology Laboratory in 1981-1983, making the Bluegrass Site one of the most thoroughly excavated Middle-Late Archaic "base-camps" in southern Indiana. The extensive samples of floral, faunal and lithic materials from the site, in conjunction with C-14 dates and human remains, provide an important data base. From it, a more comprehensive view of the Middle-Late Archaic way-of-life in southwestern Indiana and adjacent areas of the Midwest is anticipated.

Archaeology in the Coal Fields: An Update. Ruth A. Brinker, Division of Reclamation, 201 W. Main St., Jasonville, Indiana 47438.——The interactions among archaeological interests, the Division of Reclamation, and the coal mining industry have generated an exciting and sometimes confusing year of meetings, conferences,, agreements, and decisions. On the positive side, the Bluegrass Site was excavated, the Haug Sites were tested, on one coal area a sample survey was completed and effects to one site (the Drizzle Site) were mitigated, the Burger Site will be assessed and avoided by mining, one mine permit was sample surveyed, and one permit was conditioned to mitigate effects on the Kohlenlager Site. Despite efforts to encourage three companies to consider cultural resources on their mine areas, they were approved for mining without conditions. The one and only decision for an archaeological site to be declared unsuitable for mining, the Beehunter Site, was back in court. A Dubois County judge agreed that the site was important, but declaring it unsuitable was a constitutional "taking" and the State would have to pay. The decision will be appealed. Public participation in the permitting process has been very active. Eight informal conferences were held, and five led to positive results.

A petition for rule changes concerning cultural resources was approved. New rules are currently being written.

A Preliminary Analysis of Chert Tools and Debitage Collected from Sites in the Glendale Data Center. RAYMOND J. BUECHLER AND KIMBERLY A. WATKINS, Indiana State University Anthropology Laboratory, Terre Haute, Indiana 47809.——During May and June in 1987 members of the Indiana State University Anthropology Laboratory conducted a systematic survey of 1000 acres near Glendale in Daviess and Pike counties. A preliminary analysis of chert tools and debitage recovered from sites in the "data center" suggests culturally-temporally distinct patterns of chert usage occur.

Toward a Model of Chert Usage in Southwestern Indiana: A Diachronic Perspective. MARK CANTIN, Department of Anthropology, Indiana University, Bloomington, Indiana 47405.——This paper examines the differential use of cherts through time in the Wabash Lowlands Physiographic Province of southwestern Indiana. The data base consists of some 800 culturally-temporally sensitive projectile points collected from 12 "study centers" within the area. Results indicate a recurrent pattern in variability of the cultural-temporal selection of cherts, and suggest the observed variability is a reflection of an array of complex cultural systems.

Variability within Middle-Late Archaic Projectile Point Morphology and Technology: Implications for the Cultural Chronology of Southwest Indiana. MARK CANTIN, Department of Anthropology, Indiana University, Bloomington, Indiana 47405.——Projectile points characteristic of the Middle-Late Archaic in southwestern Indiana and adjacent areas of the Midwest incorporate a variety of side-notched and stemmed "types". A preliminary technological, morphological and chert-type analysis of Middle-Late Archaic points from the Bluegrass Site (12W162) and culturally-temporally related sites and/or collections from a restricted area of southwest Indiana suggests a temporal distinction occurs between these point clusters. Consequently, it is suggested that an analysis of Middle-Late Archaic points from dated base-camps would be useful for refining the Middle-Late Archaic culture chronology of southwestern Indiana.

An Earthwork Chronology for Mounds State Park: Implications for Early and Middle Woodland in East Central Indiana. DONALD R. COCHRAN, Department of Anthropology, Ball State University, Muncie, Indiana 47306.——Archaeologists have previously assumed that earthworks at Mounds State Park and other earth enclosure sites in east central Indiana were constructed within a short period of time. An alternative hypothesis of sequential construction of earthworks at the site throughout the tenure of Early and Middle Woodland culture within the region is more defensible with the regional data. The hypothesis was tested during the 1987 BSU field school by sampling three enclosures to obtain radiocarbon dates.

Rescue Excavations at the Hesher Site, A Late Woodland Cemetery in East Central Indiana. DONALD R. COCHRAN, Department of Anthropology, Ball State University, Muncie, Indiana 47306.——The Hesher site, discovered during bridge construction in Henry County, Indiana, was partially excavated in July 1987. The site contained 38 prehistoric features including 12 human burials, two dog burials, two fire pits, eight empty pits, and 14 artifact caches. Artifacts from the site indicate connections with the nearby Commissary cemetery and the Van Nuys habitation site and with Albee culture sites in the White and Wabash drainage basins of Indiana. Differences between the Hesher and Commissary cemeteries suggest chronological and/or cultural divisions.

Artificial Cranial Deformation at Angel Site, Vanderburgh County, Indiana. SHERRI L. HILGEMAN, Glenn A. Black Laboratory of Archaeology, Indiana University, Bloomington, Indiana 47405.——Artificial cranial deformation is the alteration of the natural morphology of the vault by cultural practices. Analysis of a sample of crania from the Late Prehistoric Angel site in Vanderburgh County, Indiana shows that while the overall degree of artificial cranial deformation is slighter than that in other parts of the New World, it appears to be more prevalent than at other roughly contemporaneous sites studied in the Midwestern United States. Markers of nutritional stress, porotic hyperostosis and cribra orbitalia, also seem to be correlated with the degree of artificial cranial deformation.

Archaeological Testing of the Van Duyan Site, Parke County, Indiana. MISTY JACKSON AND C. MICHAEL ANSLINGER, Indiana State University Anthropology Laboratory, Terre Haute, Indiana 47809.——Tests of the Van Duyan Site in southwest Parke County identified a Riverton Culture food-processing area. An area of intensively burned surfaces with associated fire-cracked rock and charcoal was documented around a central deposit of mussel shells. Data from intensive surface recovery and test excavation indicate that the site served as a specialized camp as described by Winters (1969) settlement model. It is suggested that resources within the local region of the Shelbyville Moraine account for differences between settlement in this area and that of the lower Wabash Valley studied by Winters.

The Archaeological Resources of the Maumee River Valley in Allen County, Indiana. JAMES A. MOHOW, Archaeological Resources Management Service, Ball State University, Muncie, Indiana 47306.——The Archaeological Resources Management Service at Ball State University conducted a sampling survey of a seven mile section of the Maumee River Valley in Allen County, Indiana. In addition to the primary survey, the project conducted an experiment in resurveying previously surveyed sample units, interviewewd local collectors, and analyzed and tabulated data from a local collection with site level provenience. The project also reevaluated data previously collected from an adjacent section of the river valley and tested four sites in the latter study area. The project recovered and synthesized a range of data essential to interpreting chronology and settlement patterns in the northeast Indiana region.

Paleopathology of an Historic Indian Burial from the Angel Site. BRIAN G. REDMOND, Glenn A. Black Laboratory of Archaeology, Indiana University, Bloomington, Indiana 47405.——In December 1940 an intrusive, historic Indian burial was uncovered in Mound F at the Middle-Mississippian Angel Site (12Vg1). A recent examination of the skeletal remains of this individual revealed evidence of a gunshot wound to the right femur. Signs of initial healing on fragments of the shattered femoral shaft indicated that the victim may have survived for a period of up to seven days after the injury occurred. Preserved fragments of cloth, bark, and wood stained with a red pigment were found in the grave and have been interpreted as the possible remains of splints used to treat the injured leg. Trace element analysis by Nelson Shaffer of the Indiana Geological Survey has identified the red pigment as mercuric oxide (HgS) or vermilion; a substance that was traded extensively during the historic period and appears to have had both symbolic and medicinal value.

Chemical Dating of Bone. MARK R. SCHURR, Glenn A. Black Laboratory of Archaeology, Indiana University, Bloomington, Indiana 47405.——Chemical methods for dating prehistoric bones can be used to determine the relative ages of burials or other archaeological features. The basic principles of chemical dating are reviewed and the applicability,

availability, and costs of nitrogen, fluoride, and trace element analyses are compared. Preliminary data testing the use of nitrogen and fluoride analyses for dating burials from the Angel Site (12Vg1) are presented to illustrate how techniques for chemical dating can be applied.

Early Paleo-Indian Lithic Exploitation in Southern Indiana; A View from the Sites. KENNETH B. TANKERSLEY, Glenn A. Black Laboratory of Archaeology, Indiana University, Bloomington, Indiana 47405.——Approximately 60 fluted points from three early Paleo-Indian sites and two site groups in southern Indiana have been petrologically examined. The geographic distribution of the lithic source areas was compared to the artifacts manufactured from those sources. The results of these analyses illustrate a pattern most closely associated with unrestricted lithic exploitation and highly mobile foraging.

Plummer, Stanford, and Scipio Cherts: Descriptions, Sources, and Utilization in Sections of the White River Valley. CURTIS H. TOMAK, Indiana Department of Highways, State Office Building, Indianapolis, Indiana 46204.——The original formal treatment of Plummer chert and Stanford chert appeared in my Masters thesis in 1970, and these cherts have been discussed in subsequent publications. Scipio is a recently defined chert type. Plummer is a blackish material which occurs naturally in the area from Spencer County north to Putnam County. Stanford chert is gray, and sources occur in Monroe and Lawrence counties. Scipio is basically a gray chert and is a noticeable constituent of collections from the Bartholomew-Decatur-Jackson-Jennings county area. Some natrual chert locations have been found, and geological contexts have been at least fairly well determined. Workshop sites at which these cherts were processed have been surveyed. Indications of the differential prehistoric utilization of Plummer and Stanford cherts are provided in part by an analysis of a sample of over 2500 points from Greene County. Once noticeable association is that between Stanford chert and Riverton points. Scipio chert was frequently used for Late Archaic artifacts, including Riverton points, as indicated by collections from the valley of the East Fork.

BOTANY

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ABSTRACTS

The Effect of Abscisic Acid on Membrane Aggregation and Fusion. BLAIR BRENGLE, WILLIAM STILLWELL AND STEPHEN WASSALL, Departments of Biology and Physics, Indiana University-Purdue University at Indianapolis, Indianapolis, Indiana 46223.——Abscisic acid (ABA) is shown to enhance the rate of aggregation of sonicated phospholipid vesicles as measured by a change in light scattering. ABA-induced aggregation was measured for phosphatidylcholine liposomes below the phase transition temperature when a second membrane component (phosphatidylethanolamine, phosphatidylglycerol, phosphatidic acid, stearylamine) was added. Pure phosphatidylcholine bilayers as well as mixed component bilayers above their phase transition remain stable in the presence of ABA. Factors upon which aggregation is dependent include the concentration of ABA, the concentration of the second membrane component, and the pH of the surrounding solution. The extent to which ABA enhances membrane fusion has been measured using spectrofluorometric assays. These findings further support our hypothesis that ABA is causing perturbations in the membrane by interacting with bilayers at the regions of interface between two different phospholipids.

Effect of Inductive Photoperiods on Cannabinoid Biosynthesis in Seedlings of *Cannabis sativa* L. (Cannabaceae). O. P. CARACCI AND C. T. HAMMOND, Department of Biology, Saint Meinrad College, St. Meinrad, Indiana 47577.——Recent interest in cannabinoid production using seedling systems prompted our investigation into the effect of inductive photosystems on cannabinoid biosynthesis. Cannabinoids found by thin layer chromatography of 6 day old seedlings grown under floral inductive photoperiods (light 8 hr/dark 16 hr) were compared with those found in seedlings grown under vegetative non-inductive photoperiods (light 16 hr/dark 8 hr). Tetrahydrocannabinol (THC) and cannabichromene (CBC) biosynthesis were found to be comparable, but a significant inhibition was seen in cannabigerol (CBG) production. Seedlings grown under inductive cycles whose leaf lengths averaged less than 4 cm showed no trace of CBG production while there was significant CBG production in non-inductive seedling control groups. Cannabidiol (CBD) is absent in our Mexican strain.

Micropropagation of Rare and Endangered *Hippeastrum* (Amaryllis) Species. ELLEN A. G. CHERNOFF, Department of Biology, Indiana University-Purdue University at Indianapolis. Indianapolis, Indiana 46223.——A joint project has been undertaken with plantsman Steve Lowe at the San Antonio Zoological Gardens to propagate rare and endangered *Hippeastrum* (formerly *Amaryllis*) species in tissue culture. Habitat in South and Central America is rapidly disappearing for many of these showy petaloid bulbous monocots. Many of the species involved are the parental stock, many generations removed, of the large hybrid "amaryllises" grown commercially by Dutch, South African, and U.S. bulb companies. The eventual aim of this project is to provide significant numbers

of virus-free plants for distribution to botanical gardens, universities, and private collections for scientific and horticultural purposes. Twelve species are being used in this initial effort. Conditions are being used that have been shown to be successful for shoot multiplication from bulb scale explants of the large commercial hybrids. The media in use are Murashige and Skoog based with 1 mg/L each 2.4-D and BAP or 1 mg/L NAA alone. The preferred growth conditions vary from species to species. Initial results show that *H ambiguum* responds well to the 2.4-D/BAP medium while *H. evansiae, H. fragrantissima*, and *H. reginae* respond best to NAA alone.

Comparison of Iron Sources for *Pilobolus*. K. Michael Foos and Judith A. Royer, Department of Biology, Indiana University East, Richmond, Indiana 47374.——In nature *Pilobolus* is restricted to growth on dung because of its absolute requirement for chelated iron. Several synthetic media have been formulated to provide which include different sources of iron for *Pilobolus*, permitting it to be cultured away from its natural source of nutrients. Each medium reported to support growth and sporulation of *Pilobolus* has been tested on only a few isolates of the fungus. We have cultivated 24 isolates of five species on media containing three forms of chelated iron reported to support its growth. *Pilobolus longpipes* could not be satisfactorily cultivated on any of the iron sources. *Pilobolus crystallinus* and *P. kleinii* exhibited variability. Some isolates of each species grew well and sporulated on each of the iron sources, and some of the isolates did not. *Pilobolus umbonatus* grew, but produced no sporangia; *P. roridus* produced sporangia irregularly. From this study it appears that multiple iron transport mechanisms may exist in *Pilobolus*. None of the three iron sources, claimed in the literature to support growth and sporulation, universally supported the growth and/or sporulation of isolates of *Pilobolus*.

In Vitro Propagation of Pin Oak. Traci Gilland and James J. Tobolski, Indiana University-Purdue University at Fort Wayne, 2101 Coliseum Blvd. East, Fort Wayne, Indiana 46805-1499.——Three shoot multiplication media were compared for in vitro propagation of pin oak (*Quercus palustris* Muenchh.). Surface sterilized modal segments of pin oak seedlings were cultured on broad-leaved tree medium (BTM), woody plant medium (WPM), and Chu N7 medium. Stem segments on WPM medium produced a few shoots in 5-6 weeks and stem segments on Chu N7 medium produced few if any shoots. BTM gave the best results with 80% of the stem segments producing several shoots in 4-5 weeks. However, only one of these shoots formed well-developed leaves and was acceptable for rooting. Rooting of cultured shoots was attempted on either a modified BTM or Chu N6 medium. The BTM rooting medium was one-half strength salt base with 10 g/l sucrose and 0.3 mg/l indolebutyric acid. Pyridoxine and amino acids were deleted. The Chu N6 medium was supplemented was 0.5 mg/l naphthaleneacetic acid, 0.1 mg/l 2, 3-5-triiodobenzoic acid and 100.0 mg/l proline. The modified Chu N6 medium produced no rooted shoots but the BTM medium had a 20% success rate. Several rooted shoots were planted in a 1:1 peat and perlite mixture. The plantlets were acclimatized for 6 weeks and then moved to the greenhouse where growth continued very slowly.

Ultrastructure of Specialized Plastids in the Glandular Secretory System of *Cannabis sativa* L. (Cannabaceae). C. T. Hammond and P. G. Mahlberg, Department of Biology, St. Meinrad College, St. Meinrad, Indiana 47577, Indiana University, Bloomington, Indiana 47405.——Secretory glands on floral axes of *Cannabis* contain cannabinoids and possess plastids structurally and functionally unlike chloro-plastids in adjacent non-glandular cells. The secretory cavity is the site of accumulation of cannabinoids and other secreted products after synthesis in gland disk cells. The plastids in the disk cells reproduce to become the dominant organellar population concommitant with timing of maximal

secretory activity. Plastids develop an extensive paracrystalline body which completely fills the mature plastid. Structurally, the paracrystalline body resembles a prolamellar body, but is much more extensive in its development and, unlike a prolamellar body, does not become transformed into a granal thylakoid system. The organization of the paracrystalline body appears to be unique to the secretary system in *Cannabis*.

A Remarkable Purple-flowered Specimen of *Daucus carota*. THOMAS R. MERTENS, Department of Biology, Ball State University, Muncie, Indiana 47306.——Deam (*Flora of Indiana*, 1940) and others have reported that the flowers of Queen Anne's-lace vary from white to yellow with some specimens having one or more purple flowers in the inflorescence. Deam also notes that rare specimens may have rose colored flowers and that such specimens have been assigned to forma *rosea* by Millspaugh. A single purple flowered plant was found in western Randolph County, Indiana in late June, 1985. All flowers on all umbels of the plant were a deep reddish-purple. Seeds were harvested from the plant in late August, 1985 and were germinated in the greenhouse in Spring, 1986. Seedlings were transplanted to the Christy Woods Arboretum on the Ball State University campus where they matured and bloomed in June, 1987. Many of these plants bore flowers that were as intensely pigmented as those of the parent plant. Two authorities consulted report never having seen *Daucus* specimens with such deeply colored flowers.

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The Effects of Inbreeding in Black Walnut

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Introduction

Black walnut (*Juglans singra* L.) is not an abundant tree species in the eastern deciduous forest, but tends to occur in groups or as scattered individuals on good sites. Since colonial times, not only have black walnut trees been harvested for manufacturing purposes, but land clearing throughout the species' natural range has reduced its domain to small, isolated tracts, often less than 40 acres in size. Thus, the remaining black walnut population has suffered a reduction in effective breeding size by virtue of reduction in the number of breeding individuals within a stand and the likely inability of trees between isolated woodlots to interbreed.

Walnut trees probably occurred every few hundred feet in most deciduous forests of the Central States as it does today in the few remaining uncut woodlots. Therefore, it is probable that gene flow could readily occur among trees within stands and between nearby stands until recent times when cutting destroyed the pattern of continuous forest.

Is pollen dispersal distance great enough to overcome the wide spatial gaps in the breeding population? Black walnut is wind-pollinated and pollen is theoretically capable of traveling long distances; however, viable black walnut pollen grains are larger and heavier than most other wind pollinated hardwood or herbaceous species (1, 14). Thus, black walnut pollen may not travel as far as the pollen of some other anemophilous species. Persian walnut pollen may be carried up to one mile from its source, but its effective distance is only 200 to 300 feet (7). Wood (15) calculated that a stigma of Persian walnut in the lower branches of a tree would receive approximately 192 pollen grains while a stigma 500 feet from the pollen source under ideal conditions would receive only 24 grains, and a stigma at one-half mile would receive no pollen.

The viability of freshly dehisced pollen from 78 individual black walnut trees over a 3 year period averaged only 34 percent and ranged from 0 to 90 percent (8). Pollen grains that fail to germinate appear to be devoid of protoplasm, and, therefore, are probably lighter in weight. These dead pollen grains would tend to carry farther from their point of origin than viable grains.

Weather conditions cause dichogamy to vary from year to year; however, overlap of pollen maturation with female flower receptivity occurred in 41 percent of 34 black walnut trees during a spring of reasonably normal weather (8). As a result of these findings, selfing could occur in natural black walnut stands.

One factor that may reduce selfing is that excessive pollen on the stigmas inhibits normal fertilization. Beineke and Masters (2) found that only 8 percent of female flowers having excessive amounts of pollen applied developed viable seed, while 66 percent of wind-pollinated flowers produced seed. Thus, female flowers below ripening catkins on the same tree would be overwhelmed by copious amounts of pollen and abscise.

Considering all of these factors, if breeding among scattered stands is not occurring, is the forced breeding among closely related individuals within a stand detrimental? Inbreeding depression has been confirmed in a wide variety of other woody plant families. For example, in the Pinaceae, all genera show growth depression and reduced vigor of inbred seedlings (6, 12). Inbreeding depression has also been found in species related

to black walnut. In pecan (*Carya illinoensis* [Wangenh.] K. Koch), inbred seedlings were 23 percent shorter than cross-pollinated seedlings produced by the same tree (10). Serr and Forde (11) found 3 of 45 selfed Payne cultivar Persian walnut (*Juglans regia* L.) seedlings were dwarfed, low in vigor, and susceptible to winter die-back. In general, the selfs had fewer desirable qualities and lower vigor than Payne crossed with other cultivars.

Selfing and related matings have been carried out in black walnut and Persian walnut (1, 15). In black walnut Beineke (1) found that forced selfing produced 22 percent seed set, while controlled-pollinated outcrosses produced 18 and wind-pollinated 49 percent seed set. In later studies, related matings (backcrosses) resulted in 62 percent seed set.

The objective of this long-term study has been to evaluate the effects of mating of closely related black walnut individuals.

Materials and Methods

In 1971, three black walnut trees in the vicinity West Lafayette, Indiana were selfed and outcrossed by controlled pollination techniques described by Beineke and Masters (2), Beineke and Masters (3), and Beineke, Masters and Pennington (4). Also, wind pollinated seed was collected from each tree. The resultant seedlings were outplanted in a progeny test in Park County, Indiana on land owned by Pierson-Hollowell Company. Survival, height, diameter, and stem form data was last obtained in 1986. Volume for each progeny was calculated according to Todhunter et al. (13).

In another experiment, an outstanding seedling was selected and replicated by grafting. When the grafts reached flowering age, pollen was collected from its maternal parent and applied to the stigmas of female flowers on the grafted seedling to produce a backcross. Seedlings were outplanted in a progeny test at Purdue University's Martell Forest near West Lafayette, Indiana.

Results

Remeasurement of the 14-year study confirms earlier speculation that inbreeding and closely related matings in black walnut reduce growth rate and vigor (1). At age 14, inbreeding depression occurred in seed germination (11 percent), height (9 percent), diameter (18 percent), volume (36 percent), and form (16 percent) as compared to outcrossed progeny; however, seedling survival of selfs after outplanting was as good as the outcrossed seedlings (Table 1).

Table 1. Inbreeding depression in selfed and backcrossed black walnut seedlings.

	Number of Seedlings	Seed Germination (Percent)	Seedling Survival (Percent)	Height (m)	DBH (cm)	Volume[1] (cu.m)	Form[2]
14-year-old self test							
Self	32	72.7	88.9	4.42	5.97	.0081	3.73
Outcross	61	81.4	88.4	4.83	7.32	.0127	3.22
Wind	98	97.9	97.0	4.80	6.93	.0115	3.57
Inbreeding depression (%)		10.7[3]	—	8.5	18.4	36.2	15.8
7-year-old backcross test							
Backcross	8	—	87.5	3.58	2.90	.0019	3.71
Female parental	10	—	100	4.46	4.45	.0048	3.60
Male parental	4	—	100	4.49	4.39	.0047	3.75
Inbreeding depression (%) (male, female mean)		—	12.5	20.1	34.5	60.3	1.0

[1] From "A Volume Equation for Seedling and Sapling Black Walnut," 1979. M. N. Todhunter, S. E. McKeand, and W. F. Beineke. Purdue Agr. Expt. Sta. Bul. No. 197. 3 p.

[2] From 1 = excellent to 5 = very poor stem form.

[3] Calculated using outcross as the basis.

In the seven-year-old backcross study, inbreeding depression was greater than was observed from selfs with the exception of form. At age seven, survival, height, diameter, and volume were depressed by 12.5, 20.1, 34.5, and 60.3 percent, respectively, as compared to wind-pollinated progeny (Table 1). Stem form was nearly the same as the male and female parental mean.

Discussion

Evidence from these experiments suggests that selfs and closely related matings produce black walnut trees of less than average growth rate and vigor. If this is occurring in our natural stands, the effect on naturally regenerated populations of black walnut could be a reduction in growth and form. As the best trees are cut leaving the worst to reproduce, and as distances between trees and woodlots increases, the detrimental effects of related matings may increase.

In forest openings on good sites, vigorous competition develops among trees, shrub, and herbaceous species. Since black walnut is intolerant, seedlings that are the product of selfing or related matings usually would be eliminated. Hence, few walnut seedlings may become established in the next generation of the forest. This could account for the paucity of walnut seedlings in forest openings with nearby seed sources. However, if inbred or related seedlings survive to maturity, further degradation of the forest could occur.

In addition, seed collected or seedling production by the state nursery systems contains a percentage of selfed seed. Since selfed seed germinates and survives nearly as well as outcrossed seed (Table 1), seedlings grown in the pampered nursery environment are eventually outplanted in plantations where every tree is expected to grow and survive to merchantable size and quality. This may be one reason significant numbers of seedlings perform poorly in most black walnut plantations.

Assuming that outcrossing occurs more frequently than inbreeding in black walnut, what is the fate of the outcrossed individual in these small isolated woodlands? The few poor-quality individuals left for breeding purposes probably perpetuate their kind; but, in addition, lacking pollination from sources outside the small, isolated woodland, the progenies will tend to become homozygous at additional loci in future generations, and undesirable genes could become fixed due to genetic drift (16).

The current interest by various agencies to bring black walnut seed orchards into production has values for reasons other than the production of genetically improved seedlings (5, 9). Black walnut seed orchards serve the vital function of providing genetic diversity that can be returned to plantations and forest openings. Contrary to popular opinion concerning the perceived threat of monoculture posed by the genetic improvement of trees, seed orchards bring together diverse genetic combinations not possible in nature. Since selections are scattered throughout a region and only one tree is selected from the same stand, related matings are unlikely events. Related matings are still possible, but careful observation and avoidance of clones that consistently overlap in flowering times should insure a minimum of selfing.

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Cyanogenesis in Woody Ornamentals

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Introduction

Many plants have the ability to synthesize compounds which are capable of liberating hydrogen cyanide upon hydrolysis. This phenomenon, known as cyanogenesis, has been recognized for more than a century and is not restricted to a particular group of plants. Cyanogenesis has been reported in bacteria, fungi, ferns, gymnosperms, and angiosperms. Among higher plants, these compounds are generally restricted to the leaves but may also occur in other plant organs. Presently cyanide has been reported from more than 2050 species of plants representing about 110 families (Seigler, 1981).

Cyanogenesis is caused by the breakdown of cyanogenic glycosides or cyanolipids in the presence of specific enzymes (β-glucosidases). The amount of cyanide produced depends on several intrinsic (genetics, part of plant, age of plant, and sometimes the sex of the plant) and extrinsic factors (climate, moisture supply, soil fertility, and frost damage) (Kingsbury, 1964). More than 30 different cyanogenic compounds have been reported, but specific compounds have been isolated from less than 200 species (Seigler, 1977). The present study was undertaken to determine the extent of cyanogenesis in common woody ornamentals in the midwestern United States because these compounds may be useful as taxonomic characters, and both the compounds and the plants that contain them are poisonous to both humans and livestock.

Materials and Methods

More than 1000 ornamental woody specimens, representing 37 species, were tested for the presence of cyanogenic compounds, as were herbarium specimens of these species in the Stover Herbarium at Eastern Illinois University (EIU). Each of these specimens were tested for the presence of hydrogen cyanide by the method previously described by Feigl and Anger (1966) and modified by Tantisewie *et al.* (1969). To conduct this test, a small amount of plant material (about 200 mg) is crushed, placed in a vial, and moistened with distilled water. A strip of filter paper impregnated with copper ethylacetoacetate and tetra base (4,4 '-tetramethyldiaminodiphenylmethane) is added to the vial so as not to touch the sample, and the vial sealed with a cork. The presence of cyanide is indicated if the filter paper turns a blue color within a 24-hour period. A negative test indicates the specimen does not contain a cyanogenic compound or lacks the enzyme capable of hydrolyzing the cyanogenic compound, or both.

A quantitative determination of cyanide was not made. An indication of the amount of cyanide produced, however, can be made by observing the extent of the color change of the Feigl Anger paper (Dickenmann, 1982). In this study the reaction was considered weak if only part of the paper turned a light blue, moderate if it turned a light to medium blue, and strong if the paper turned a deep blue. According to Dickenmann (1982), the weak reaction contains approximately 2-20 mg of HCN per kg fresh weight, the moderate reaction produces 21-50 mg of HCN per kg fresh weight, and the strong reaction produced more than 50 mg of HCN per kg fresh weight. Voucher specimens are deposited in the Stover Herbarium of Eastern Illinois University (EIU). Nomenclature follows Bailey (1964).

Results and Discussion

The species examined are discussed below, while Table 1 lists all of the species ex-

TABLE 1. Woody ornamentals which tested positive for cyanogenesis during the present study. Also included for each species is the strength of the reaction, the plant part tested, the number of individuals examined, the number of individuals that tested positive for cyanogenesis, and a reference for species that have been reported previously as cyanogenic.

Species	Speed of the reaction[1]	Strength of the reaction[2]	Plant part tested[3]	Number tested	Number cyanogenic	Reference
Aceraceae						
Acer platanoides	S	W/M	F	14	11	This Study
Acer rubrum	S	W	F	9	1	This Study
Apocynaceae						
Vinca minor	S	W	L	45	1	This Study
Buxaceae						
Buxus microphylla	S	W/M	L	10	7	This Study
Calycanthaceae						
Calycanthus floridus	S	M	L	5	1	van Valen (1978)
Celastracaea						
Euonymus alatus	S	W/S	L	55	14	This Study
Magnoliaceae						
Liriodondron tulipifera	F	S	L	120	119	van Valen (1978)
Magnolia acuminata	S	S	P	1	1	This Study
Magnolia soulangeana	S	M	P	3	3	This Study
Magnolia stellata	S	W	P	1	1	This Study
Magnolia virginiana	—	—	P	7	0	—
Oleaceae						
Syringa vulgaris	S	W/S	F	12	5	This Study
Platanaceae						
Platanus acerifolia	F	S	L	23	23	Greshoff (1909)
Platanus occidentalis	F	S	L	158	130	Greshoff (1909)
Rosaceae						
Amelanchier arborea	F	W/S	L	66	61	Seigler (1976)
Amelanchier humilis	F	S	L	8	7	Gibbs (1974)
Amelanchier interior	F	S	L	1	1	This study
Amelanchier laevis	F	S	L	4	4	Gibbs (1974)
Chaenomeles japonica	F	S	L	10	9	Rosenthaler (1926)
Cotoneaster acutifolia	F	W/S	L	10	6	Gibbs (1974)
Cotoneaster adpressa	F	S	L	1	1	Tidwell, *et al.* (1970)
Cotoneaster apiculata	F	S	L	2	2	Tidwell, *et al.* (1970)
Cotoneaster divaricata	F	S	L	16	15	Tidwell, *et al.* (1970)
Exochorda racemosa	F	S	L	1	1	Hagnauer (1971)
Kerria japonica	S	W	L	10	1	Plouvier (1948)
Malus floribunda	F	S	S	30	28	This Study
Prunus persica	F	S	L	8	8	Kingsbury (1964)
Prunus serrulata	F	S	L	1	1	This Study
Prunus triloba	F	S	L	5	5	Rosenthaler (1929)
Sorbus aucuparia	F	S	L	5	5	Seigler (1976)
Saxifragaceae						
Hydrangea arborescens	—	—	L	150	0	—
Taxaceae						
Taxus cuspidata	F	M/S	L	30	9	Hegnauer (1959)
Taxodiaceae						
Metasequoia glypostroboides	S	W/M	L	3	2	van Dijk, *et al.* (1974)
Tiliaceae						
Tilia americana	S	W/S	L	171	7	This Study
Tilia cordata	S	W/S	L	23	4	This Study
Tilia euchlora	S	W	L	3	1	This Study
Tilia tomentosa	S	M	L	3	1	This Study

[1] The speed of the reaction was considered fast (F) if less than two hours were required to give a strong test, and slow (S) is more than two hours were required.

[2] The strength of the reaction was weak (W) if the Feigl Anger paper turned a light blue, moderate (M) if it turned a medium blue, and strong (S) if it turned a deep blue.

[3] Plant part tested: L = young leaves; S = seeds; F = flowers; P = petals.

amined, the speed and strength of the cyanide test, the part of the plant examined, the number of individuals of each species examined, and the number of individuals of each species that tested positive for cyanogenesis.

Aceraceae: No member of this family has been reported previously as being cyanogenic (Tjon Sie Fat, 1979; Gibbs, 1974). During the present study, the tests of vegetative material were always negative, but the flowers of *Acer platanoides* L. (Norway maple) and *A. rubrum* L. (red maple) occasionally gave a weak to moderate positive cyanide test. Of the fourteen trees of Norway maple examined, flowers from eleven tested positive, as did flowers from one of the nine trees of red maple.

Apocynaceae: At least six members of this family have been reported to be cyanogenic (Gibbs, 1974; Seigler, 1976; Tjon Sie Fat, 1979). In addition, during the present study, of the 45 individuals of *Vinca minor* L. (small periwinkle) examined, the young leaves of one individual gave a weak positive test for cyanide.

Buxaceae: No reports of cyanogenesis have been found for this family (Gibbs, 1974; Tjon Sie Fat, 1979). Of the ten plants of *Buxus microphylla* Sieb. & Zucc. (Korean box) examined, seven gave a weak to moderate reaction within 24 hours when young leaves were tested.

Calycanthaceae: Some members of this family have been reported to be cyanogenic (Hegnauer, 1958; van Valen, 1978). During the present study, of the five individuals of *Calycanthus floridus* L. (Carolina allspice) examined, the young leaves of one gave a moderate positive test within 24 hours.

Celastraceae: Except for a single report (Tjon Sie Fat, 1979), no positive cyanide tests have been reported from members of this family. During the present study, weak to strong reactions were obtained for young leaves from 14 of the 25 cultivated specimens of *Euonymus alatus* (Thunb.) Sieb. (winged wahoo) examined. In a naturalized population of this species, found in a wooded ravine one mile east of Charleston, Coles County, Illinois, 30 individuals tested negative for cyanogenesis.

Magnoliaceae: Three species in this family have been reported as cyanogenic (Tjon Sie Fat, 1979). During the present study it was found that immature leaves of *Liriodendron tulipifera* L. (tulip tree) are cyanogenic with 119 of 120 individuals testing strongly positive, usually within one hour. Also, the petals of one individual of *Magnolia acuminata* L. (cucumber magnolia) gave a strongly positive test for cyanide, the petals of three individuals of *M. soulangeana* Soul. (flowering magnolia) gave a moderately positive test; while the petals of one individual of *M. stellata* (Sieb. & Zucc.) Maxim. (starry magnolia) gave a weakly positive test. In all three of these species, the vegetative and other floral parts gave a negative test. Also, it was not uncommon for petals from different flowers on the same plant to test negative while others gave a positive reaction. Of the seven specimens of *M. virginiana* L. (sweet bay magnolia) studied, all plant parts tested negative for cyanide.

Oleaceae: Tjon Siet Fat (1979) and Seigler (1976) list no members of this family have been reported to be cyanogenic. During the present study, flowers of *Syringa vulgaris* L. (common lilac) were found to occasionally release cyanide. Of the twelve white flowering individuals examined, five gave a weak to strong positive reaction.

Platanaceae: Five species of the genus *Platanus* are known to be cyanogenic (Fikenscher and Ruijgrok, 1977). During the present study it was found that the immature leaves of *Platanus occidentalis* L. (American sycamore) are usually strongly cyanogenic with 130 of 158 individuals testing positive. Of the 23 individuals of *P. acerifolia* (Ait.) Willd. (London plane tree) tested, all were strongly cyanogenic. Commonly, mature leaves and leaves from herbarium specimens of these two species test negative for cyanide production.

Rosaceae: More than 160 species in this family have been reported to be cyanogenic (Gibbs, 1974; Tjon Sie Fat, 1979). In many species, only the leaves are cyanogenic but

sometimes flowers, fruits, or seeds are known to release hydrogen cyanide. Most members tested during the present study were strongly cyanogenic (Table 1). All species of *Amelanchier* (juneberry), *Cotoneaster* (Cotoneaster), and *Prunus* (peach and cherry) tested were strongly cyanogenic, as were *Chaenomeles japonica* (Thunb.) Lindl. (Japanese quince), *Exochorda racemosa* (Lindl.) Rehd. (pearl-bush), *Sorbus aucuparia* L. (European mountain ash), and the seeds of *Malus floribunda* Sieb. (showy crab-apple).

Saxifragaceae: Cyanogenesis is fairly common in this family, many species of currants and gooseberries (Ribes) and hydrangea (*Hydrangea*) have been reported to produce cyanide (Tjon Sie Fat, 1979). Of the 150 individuals of *Hydrangea arborescens* L. (wild hydrangea) tested, none were cyanogenic.

Taxaceae: *Taxus cuspidata* Sieb. & Zucc. (Japanese yew) has been reported cyanogenic by Hegnauer (1959) and Towers, McInnes and Neish (1964). In the present study, of the 30 individuals examined, nine gave a moderate to strong positive reaction in less than two hours.

Taxodiaceae: *Metasequoia glypostroboides* Hu & Cheng (dawn redwood) has been reported previously to be cyanogenic (van Dijk, *et al.*, 1974). In the present study two of the individuals tested gave a weak to moderate reaction.

Tiliaceae: Gibbs (1974) reported that seven species in this family are known to be cyanogenic. In the present study four species of basswoods (*Tilia*) were found to occasionally liberate cyanide. In all four of these species cyanogenesis is sporadic and the majority of the individuals examined tested negative. In *Tilia americana* L. (American linden) only 7 of 171 individuals tested positive, in *T. cordata* Mill. (small-leaved linden) only 4 of 23 individuals were positive, while in *T. euchlora* K. Koch. (Crimean linden) and *T. tomentosa* Moench. (white linden) one of three individuals tested positive for each species.

The present study indicates that cyanogenesis is relatively common in many families of plants. Most of the taxa examined are from families in which cyanogenesis is considered to be fairly common (Gibbs, 1974; Tjon Sie Fat, 1979). Included in this group are the Apocynaceae, Calycanthaceae, Magnoliaceae, Platanaceae, Rosaceae, Taxaceae, and Tiliaceae. As more taxa of these families are examined it is very possible that cyanogenesis will be found in many of the species, particularly if numerous specimens are examined. In contrast, some of the taxa found to be cyanogenic in the present study are from families in which no previous reports of cyanide production could be found (Aceraceae, Buxaceae, and Oleaceae). It is possible that cyanogenesis is rare in these families, or equally possible that few members of these families have been tested.

The data also suggests that cyanogenesis is relatively common in ornamental woody plants. In many species, however, the extent of cyanogenesis is highly variable, as not all individuals of a taxon gave positive results (Table 1). In some taxa, particularly *Acer platanoides, Buxus microphylla, Liriodendron tulipifera, Platanus* spp., and many members of the Rosaceae, the large majority of the individuals examined tested positive for cyanide production. In other species, however, the number of cyanogenic individuals was relatively low, particularly when many specimens were available for testing.

A lethal dose of cyanide for humans is generally considered to be in the range of 0.5-3.5 mg/kg of body weight. Many of the plants tested during the present study produce high concentrations of HCN, as indicated by the deep blue color of the Feigl Anger paper. According to Dickenmann (1982) the deep blue reaction indicates that more than 50 mg of HCN are produced per kg of fresh weight. Therefore, of the taxa studied, all that gave a fast, strong reaction (Table 1) produced enough HCN to kill a human if ingested in sufficient quantities.

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The Effect of Diamide and Buthionine Sulfoximine on Glutathione Pools and Transmembrane Electron Transport by Cultured Carrot Cells

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Introduction

The role of glutathione, a tripeptide (γ-L-glutamyl-L-cysteinylglycine) is well established in the sulfur cycle as a storage and transport form for reduced sulfur (10). It also functions in detoxification of pesticides by plants (10), but its role as a possible internal electron donor for transplasma membrane electron transport has not been studied previously. This study was undertaken with the purpose of establishing whether glutathione had an effect on plasma membrane redox reactions of cultured carrot cells. A link between electron transport and glutathione content of cells was established by decreasing the internal glutathione concentration. This was accomplished by growing cells in presence of diamide (7), a chemical known to oxidize the reduced form of glutathione (GSH) to its oxidized from (GSSG) and buthionine sulfoximine, a glutathione biosynthesis inhibitor (6).

Materials and Methods

Carrot cells (*Daucus carota* L.) were grown for 8 days in liquid suspension culture on Murashige and Skoog's medium without agar as previously described (2). Control cells received no diamide or buthionine sulfoximine but 3 replications each received 0.2, 0.4, 0.6 and 0.8 mM diamide or 100, 200 or 300 μM buthionine sulfoximine immediately after the initial transfer of cells on day 1. After inoculation, the flasks were placed on a gyrating shaker at 24 °C under room lights. On day 8, cells were harvested by centrifugation in a table model non-refrigerated International centrifuge at setting 4 (200 $\times$ g) for 2 min. For assaying plasma membrane redox reactions or H + excretion, cells were washed twice with sucrose-salts solution (0.1 M sucrose with 10mM each NaCl, KCl and $CaCl_2$) and suspended in 50 ml of the same. For extracting glutathione from cells, they were washed twice with distilled water and stored frozen until used.

For dry weight determination, 1 ml aliquots of cells in triplicate were washed with distilled water and placed in a 100 °C oven overnight.

Plasma membrane electron transport was measured as ferricyanide reduction in an Aminco DW-2A double beam spectrophotometer as the difference between 420 nm (measuring wavelength) and 500 nm (reference wavelength), following the reduction of ferricyanide. The reaction mixture contained 25mM Tris-Mes, pH 7, sucrose-salts and 0.1 ml cells (about 5μg dry wt.). 100μM ferricyanide was added to start the reaction. Ferricyanide reduction rates were calculated, using a millimolar extinction coefficient of 1.

Proton excretion by carrot cells was measured with a pH meter equipped with a Corning combination pH electrode in a water-jacketed chamber at 24 °C. Air was bubbled through the chamber's sidearm continuously to dissipate CO_2. The reaction mixture contained 4.9 ml sucrose-salts, 25μM phosphate buffer, pH 7, and 0.1 ml cells (about 5μg dry wt.). After measuring the basal rate for 5 min., 100μM ferricyanide was added to measure the excretion of redox-driven protons. All rates were recorded with a Linear recorder. Proton excretion rates were calculated from adding known amounts of HCl at the end of each assay.

Glutathione (GSH in combination with GSSG) was extracted by the method of Aker-

boom and Sies (1), except the NEM step was omitted. Frozen cells (2.5-3g wet wt.) were ground in 3 ml of 10% perchloric acid with a mortar and pestle. The extract was filtered through Miracloth and the residue reground in 7 ml perchloric acid solution. The combined acid extracts were centrifuged at 5000 × g for 5 min to remove the precipitated proteins. The supernatant, containing the extracted mixture of reduced and oxidized forms of glutathione and mixed acid-soluble disulfides, was neutralized with a few drops of 2 M NaOH dissolved in 0.3 M Tris-Mes buffer. The spectrophotometric assay for reduced and oxidized glutathione was performed immediately after neutralization of the extract. The reaction mixture contained 1 ml buffer (0.1 M potassium phosphate, pH 7, with 0.001 M EDTA, 0.1 ml neutralized extract, 50μl NADPH of 4mg/ml stock, dissolved in the above buffer, and 20μl glutathione reductase (6 units per ml), dissolved in buffer. The reaction was started by the addition of 20μl DTNB (1.5 mg dissolved in 0.5% $NaHCO_3$). The reaction rates, seen as an increase of absorbance of 412 nm, were recorded with a Linear recorder for 3 min. At the end of the assay, the absorbance at 412 was taken manually as a single absorbance reading. The reaction rates for the recorded assay were calibrated from known amounts of glutathione added to the reaction mixture in place of the neutralized carrot cell extract. The glutathione concentration from a single absorbance reading at 412 nm after a 3 min incubation was obtained by dividing the O.D. per min at 412 nm by the extinction coefficient for the DTNB product A_{412nm} = 13.6mM) and by the dry weight of cells used per assay.

Results and Discussion

The role of glutathione in plant cells has been reveiwed by Rennenberg (10) and in animal cells by Kosower and Kosower (7) and by Meister (8). In green plants glutathione serves as the main long-distance transport form of sulfur from the leaves to the roots and as a storage form of sulfur. Glutathione also participates in the detoxification of hydrogen peroxide and fungicides. According to Reese and Wager (9), buthionine sulfoximine, an inhibitor of glutathione biosynthesis, causes a decrease in the levels of a cadmium-binding peptide, which leads to reduced growth of cultured tobacco cells. Recently it has been shown by Earshaw and Johnson (5) that the addition of 0.1 mM glutathione to wild carrot suspension cultures caused these cells to proliferate whereas the addition of 0.3 mM buthionine sulfoximine, which decreased cellular glutathione levels by inhibiting the action of γ-glutamylcysteine sythetase (6), promoted the generation of somatic embryos. Since Crane et al. (4) had shown previously that plasma membrane electron transport reactions were associated with growth of cultured cells, it was decided to find out what effect glutathione levels in carrot cells had on transmembrane electron transport.

As shown in Table I, adding diamide to growing carrot cells decreased the internal glutathione content to a maximum 23% by oxidation of blutathione (8). Buthionine sulfox-

Table 1. The Effect of Diamide and Buthionine Sulfoximine on Total Gluthathione Levels of Cultured Carrot Cells.

Treatment	Gluthatione (GSH + GSSG) (μmoles g dry wt.$^{-1}$)	Decrease (%)
None	2.21	—
0.2mM diamide	2.20	0
0.4mM diamide	1.70	23
0.6mM diamide	1.85	16
0.8mM diamide	1.80	18
None	2.54	—
100μM buthionine sulfoximine	1.41	44
200μM buthionine sulfoximine	0.51	80
300μM buthionine sulfoximine	0.18	93

imine, an inhibitor of γ-glutamylcysteine synthetase (10) proved to be more effective than diamide in reducing the internal glutathione levels in carrot cells with a decrease to 93% of control cell level (Table I). Oxidation of reduced glutathione by diamide and a block of its biosynthesis by buthionine sulfoximine affected plasma membrane redox in a similar manner by causing 25 to 37% inhibition of ferricyanide reduction rates (Fig. 1, A and

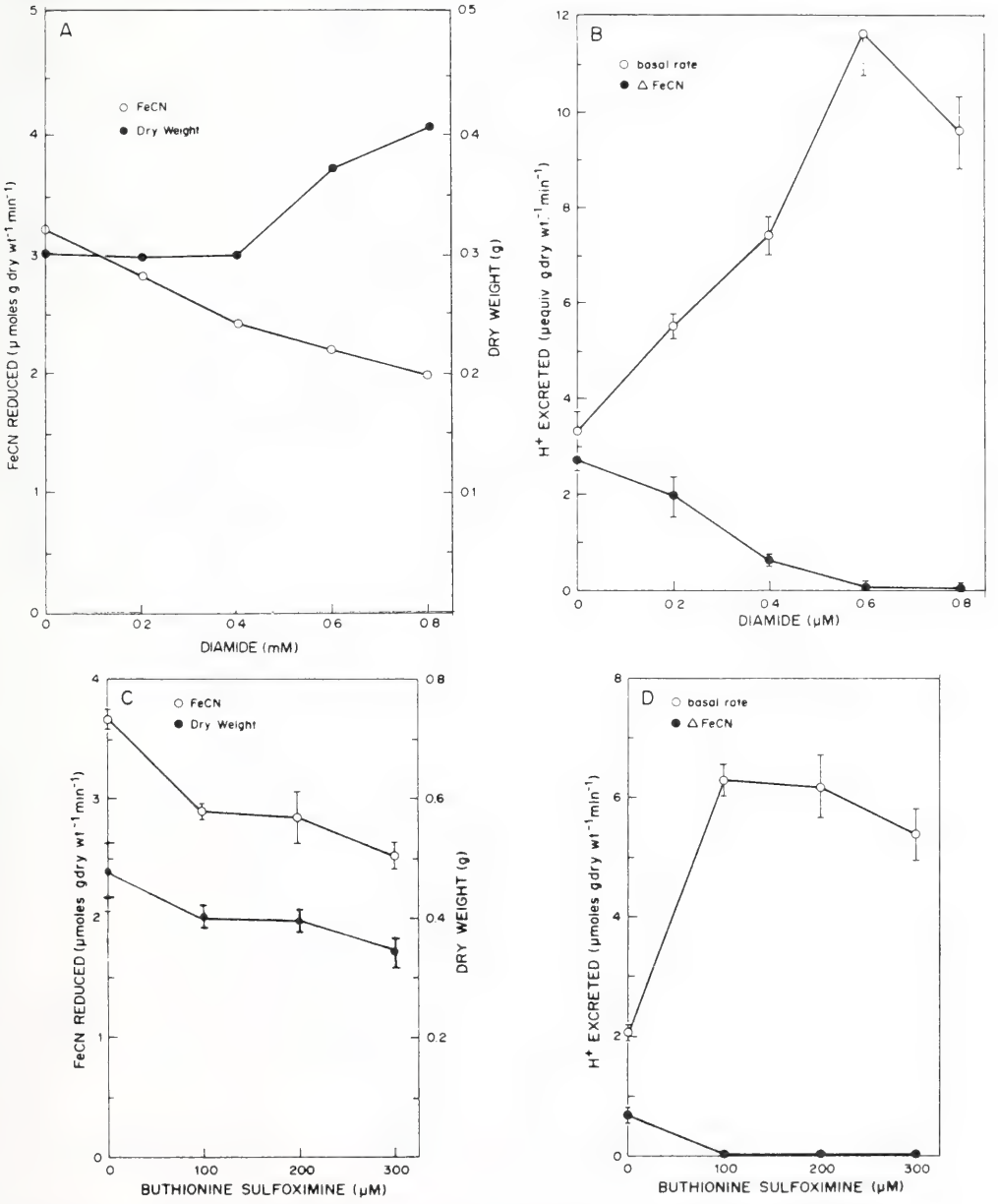

FIGURE 1. The effect of growing carrot cells with inhibitors. A). Transmembrane ferricyanide reduction rate and dry weight of carrot cells grown for 8 days with various concentrations of diamide. B). Proton excretion by carrot cells grown for 8 days with various concentrations of diamide. C). Transmembrane ferricyanide reduction and dry weight of carrot cells grown for 8 days with various concentrations of buthionine sulfoximine. D). Proton excretion by carrot cells grown with various concentrations of buthionine sulfoximine. Reaction conditions for each assay as described in the Materials and Methods section. The H + excretion rate with ferricyanide is the net rate after subtraction of the basal rate.

1, C). On the other hand, these agents produced different effects on cell proliferation. Diamide (0.8mM) stimulated carrot cell growth, while buthionine sulfoximine inhibited growth 30%. The proton excretion patterns (Fig. 1, B and 1, D) were similar after treatment with both compounds. The basal rate of proton excretion, attributed mainly to the action of the plasma membrane H+ -ATPase, was stimulated 3-fold by both compounds. Proton excretion due to plasma membrane electron transport in presence of potassium ferricyanide was inhibited 100% in both cases.

Since green tobacco cells cultured under photoheterotrophic conditions are known to release 30× more glutathione (up to 3.5mM) into the culture medium than cells devoid of chlorophyll (10), it can be argued that transmembrane ferricyanide reduction in such cells may be due to the release of glutathione. In our studies with carrot cells a rapid rate of glutathione release is not likely because chloroplasts are absent from the yellow callus cells and the small amount of glutathione released is too slow to account for the ferricyanide reduction rates observed (about 4 μmoles g dry wt.$^{-1}$ min $^{-1}$). We have shown that release of reducing agents from carrot cells is less than 10% of the ferricyanide reduction rate (3).

In summary, the present data indicates that growing carrot cells for 8 days with diamide, an oxidizing agent, and buthionine sulfoximine, an inhibitor of glutathione biosynthesis, result in decreased levels of glutathione in the cells, which leads to a partial inhibition (up to 37%) of plasma membrane redox reactions and 100% inhibition of oxidant associated proton excretion, while the proton excretion by the plasma membrane H+ -ATPase is stimulated 3-fold with both treatments. The difference in growth responses may be attributed to the difference in action of the reagents. Diamide causes oxidation of reduced glutathione, whereas buthionine sulfoximine causes loss of both oxidized and reduced forms.

Acknowledgment

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A Compilation of Plant Diseases and Disorders in Indiana—1987

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Introduction

The Plant Diagnostic Clinic in the Department of Botany and Plant Pathology at Purdue University is a service of the Cooperative Extension Service, Purdue Agricultural Experiment Station. Plant disease diagnosis and weed identification are gratuitous services offered by the clinic. Approximately 70% of the samples submitted annually come from county extension agents. The remainder come directly from commercial growers, homeowners, private consultants and other interested persons. This paper is a summary of the major plant diseases and disorders which were diagnosed in the clinic and throughout the state in 1987.

Methods

Plant specimens are submitted to the Plant Diagnostic Clinic from county extension agents, homeowners, growers, nursery operators, consultants, and others. Specimens are diagnosed visually or by culturing the pathogen on selected media. Some virus diseases are diagnosed serologically and others by the leaf dip (negative stain) technique utilizing the electron microscope. Once a disease or disorder is diagnosed, appropriate control measures are suggested. A summary of the samples diagnosed from January 1 through November 25, 1987 is given in Table 1.

Results

As of November 25, 1987 the clinic had received 1105 samples, including ornamentals, fruits, vegetables, turf and agronomic crops. Of these samples approximately 50% were diagnosed as infectious disease problems, 23% were diagnosed as environmental disorders, 6% were diagnosed as chemical injury, and 4% exhibited a nutritional problem. The remaining samples were either damaged by insects, were poor samples or lacked adequate information for a proper diagnosis.

In general, temperatures were above normal and precipitation was below normal for most areas of the state during the entire growing season. Warm dry weather in May and June prevented serious disease epidemics from occurring on spring crops. However, localized areas of the state received sporadic thunderstorms in July, with some of these storms producing up to 8 inches of rainfall in a 24 hour period. Disease conditions were directly related to these weather patterns. The wet weather in the latter part of the growing season did promote severe disease outbreaks on several main season and late season crops.

Shade and Ornamental Trees

Diseases: Apple scab (*Venturia inaequalis*) of crabapples was very light in early spring due to dry weather, however, symptoms were evident by late spring and continued to increase in severity throughout the growing season. Anthracnose (*Gloeosporium* sp.) of sycamore and ash was light to moderate in most areas of the state, resulting in some minor defoliation during late May and early June. Anthracnose of white oak and hard maple was negligible. Rust (*Gymnosporangium* sp.) on hawthorn and crabapples was

Table 1. Plant samples received in the Purdue Plan Diagnostic Clinic Jan 1 through Nov 25, 1987.

Plant Samples	Number of[1] Samples	Diseases[2]	Disorders[3]	Chem.[4]	Nutr.[5]	Insect[6]	Other[7]
AGRONOMIC							
Corn	91	55	24	17	5	8	22
Soybeans	179	130	13	7	15	4	22
Small Grain	35	25	7	0	0	0	12
Forage Grasses and Legumes	24	15	2	4	1	8	4
ORNAMENTAL							
Trees-Shade and Ornamental	329	89	123	16	7	73	89
Shrubs and Groundcover	86	33	31	7	1	15	21
Flowers	73	42	5	5	4	5	16
House plants	11	2	4	0	0	1	3
FRUIT							
Tree Fruit	84	49	13	2	1	17	23
Small Fruit	45	29	7	1	1	4	10
VEGETABLE	90	44	18	6	5	4	24
TURFGRASS	58	45	9	2	0	7	11
WEED IDENTIFICATION	163	—	—	—	—	—	—
TOTAL	1268	558	256	67	40	146	248

[1] The number of diagnosed problems add up to more than the number of samples received since many samples may have more than one problem.

[2] Problems caused by an infectious disease causing agent, e.g. fungus, bacterium, virus, mycoplasma, nematode.

[3] Problem caused by noninfectious environmental stress, e.g. wind, drought, heat, soil compaction.

[4] Problem caused by herbicide/pesticide misuse.

[5] Problem caused by a nutrient imbalance.

[6] Problem caused by an insect. Does not include samples submitted to Entomology Diagnostic Clinic.

[7] "Other" includes the causal agent categories: No disease, and inadequate sample for diagnosis.

almost nonexistent due to the early dry conditions. Diplodia tip blight (*Sphaeropsis* sp.) continued to be of common occurrence in Austrian, red and scotch pine plantings. Verticillium on maple and redbud, Dutch Elm Disease (*Cerotocystis ulmi*), Rhizosphaera on spruce, and tatters on oak, a still undiagnosed malady, were prevalent. Ash yellows, a disease caused by a mycoplasma, was recognized in northern Indiana and 2 southern counties in Indiana (9) and confirmed by Dr. Sinclair of Cornell University. The extent and importance of ash yellows in Indiana is not yet known. White pine root decline (*Verticicladiella* sp.) was also diagnosed.

Disorders: Decline of maples continues to be the most common shade tree disorder. Decline was again most prevalent on older street trees planted in restricted sites and subjected to a variety of urban stress factors, eg. road salts, soil compaction, air pollutants, etc. Tree decline was also common on hickory, oak and ash. Late season leaf scorch due to extended drought periods was common. Iron chlorosis of pin oak remains the most frequently recorded nutritional disorder. Exposed buttress roots on deciduous trees, particularly silver maple, as well as loss of normal needle color on spruce were common environmental-site related problems.

Ornamentals

Disease: There were no widespread diseases of major occurrence on ornamentals. As in previous years, powdery mildew (*Erysiphe* sp.) was severe during late summer on lilac,

zinnia, dahlia and numerous other susceptible hosts. Kabatina twig blight was again diagnosed throughout Indiana (7,8). Alternaria and Botrytis blights on zinnia and marigold were frequently diagnosed. Sclerotinia damping off on ajuga, a ground cover, was a severe problem in several nurseries. Phoma tip dieback on vinca and Volutella leaf and stem blight on pachysandra were prevalent.

Disorders: Cold injury was prevalent on cotoneaster throughout middle and northern Indiana. Winter desiccation (browning) of junipers and other evergreens was common as in previous years, but not severe (1,2,3,4,5,6,7,8). A wilting and stunting of commercially field-grown statice was diagnosed as root girdling and compaction.

Tree Fruits

Disease: Early season apple scab (*V. inaequalis*) and cedar apple rust (*Gymnosporangium juniperi-virginianae*) were of minor occurence due to early dry spring weather. Fire blight (*Erwinia amylovora*) of pear and apple was severe in localized areas of southern Indiana but was not widespread, as experienced the previous year (1986) (8). Fire blight was confined to the extreme southern portion of the state. Very little blight was reported in middle and northern Indiana. Above normal wet periods during summer resulted in sooty blotch (*Gloeodes pomigena*) and fly speck (*Microthyriella rubi*) diseases of apple fruit being extremely common throughout the state. Brown rot of cherry (*Monilinia fructicola*) was prevalent at harvest, especially in northern areas of the state.

Disorders: Hail damage was the most noticeable disorder. The extent of hail damage was widely variable depending on orchard location. Extended summer drought periods resulted in scattered reports of death of newly planted trees and older stressed trees. Bitter pit on apples, a calcium deficiency/site stress/weather related problem was again diagnosed on red delicious and jonathans (6,7,8).

Small Fruits

Disease: Severe outbreaks of orange rust (*Gymnoconia peckiana*) of blackberries and raspberries were reported for individual plantings in both southern and northern Indiana. Strawberry leaf diseases (*Dendrophoma* sp.; *Diplocarpon* sp.) were prevalent during late summer and early fall. Black rot (*Guignardia bidwellii*) on grape was frequently diagnosed on samples from homeowners.

Disorders: There were no prominent disorders recorded for small fruits.

Vegetables

Crucifers: Black rot (*Xanthomonas campestris*) continues to concern growers. Not many fields of cabbage, cauliflower or broccoli were affected by this disease, but the few that were affected suffered severe losses. Brown bud, a physiological disorder that causes decay of broccoli heads was particularly prevalent among fall crops in the northern part of the state. For the first time in 6 years, brown bud also was observed on cauliflower. Black leaf spot (*Alternaria brassicae*) remains a serious problem to cauliflower growers. Most growers avoided economic loss by following a diligent (and expensive) fungicide spray program. Incidence of leaf spot on cabbage and broccoli was very low. White rust (*Albugo candidans*) was identified on spinach and mustard greens in the northern part of the state.

Cucurbits: The usual contigent of diseases on muskmelon and watermelon prevailed in 1987 (1,2,3,4,5,6,7,8). Fusarium wilt (*Fusarium oxysporum*) gummy stem blight (*Didymella bryoniae*), and anthracnose (*Colletotrichum orbiculare*) occurred with moderate frequency. Alternaria leaf blight (*Alternaria cucumerina*) remains the greatest threat to muskmelon production. Successful growers managed the disease through a combination of cultural practices and a diligent fungicide spray program. Bacterial wilt (*Erwinia*

tracheiphila) occurred on muskmelons in low frequency because of sound insect management tactics used by Indiana farmers. Powdery mildew (*Sphaerotheca fuliginea*) was identified on June 19 in a commercial muskmelon field in southwestern Indiana. Throughout the summer, specimens of mildew infected leaves were examined microscopically to determine whether the mildew species was *S. fuliginea* or *Erysiphe chicoracearum*. For all 31 samples from various cucurbits in different parts of the state, the pathogen species was identified as *S. fuliginea*. (With the aid of KOH, fibrosin bodies (small crystals) are visible in the *S. fulginea* conidia while conspicuously absent in the *E. chicoracearum* conidia, thus allowing for positive diagnosis of the powdery mildew genus.) Downy mildew (*Pseudoperonospora cubensis*) was not observed on cucurbits in 1987. Root knot nematode (*Meloidogyne incognita*) was diagnosed in 4 muskmelon and 3 watermelon fields in Knox and Gibson counties. Zucchini yellow mosaic virus was identified in several muskmelon, squash, and pumpkin fields. The disease appeared to spread quickly and caused economic yield loss in an early muskmelon field and a late pumpkin field. Pumpkins were also infected with powdery mildew, black rot, and anthracnose diseases. Pickling and slicing cucumbers remained almost disease-free, except for bacterial wilt and an unusual amount of anthracnose in varieties previously considered resistant. Non-infectious disorders of cucurbits such as injury from triazine herbicide carry over, magensium deficiency, manganese toxicity, and molybdenum deficiency also were identified.

Tomatoes: Southern bacterial wilt (*Pseudomonas solanacearum*) was a major concern among processing tomato growers. Bacterial canker (*Clavibacter michiganense* var. *michiganense*) was particularly severe among several varieties of commercially grown fresh market tomatoes. Bacterial speck (*Pseudomonas syringae* var. *tomato*) occurred with greater than usual frequency on irrigated tomatoes in the southern third of the state. Wet weather in the middle-eastern portion of the state promoted severe outbreaks of anthracnose (*Colletotrichum coccodes*) on tomato fruit, especially in fields harvested in mid-September. Tomato early blight (*Alternaria solani*) caused no severe problems, and late blight (*Phytophthora infestans*) was not diagnosed in Indiana for the fifth consecutive year. Septoria leaf spot (*Septoria lycopersici*) was the foliar disease of major concern in commercial and home garden tomatoes.

Beans: White mold (*Sclerotinia sclerotiorum*) caused a few problems on green beans grown for processing. Bean rust (*Uromyces phaseoli*), common blight of beans (*X. campestris* var. *phaseoli*), and halo blight (*P. syringae* var. *phaseolicola*) were diagnosed on late season, fresh market beans. Rust and white mold problems are expected to continue as bean acreage increases in our state.

Peppers: Bacterial spot (*Xanthomonas campestris* pv. *vesicatoria*) was presumed to be introduced into bell pepper fields in Indiana by infected transplants shipped from the southern states. The cultivar Jupiter was severely affected; many Jupiter crops were total losses this year due to bacterial spot alone. Other varieties in adjacent fields were affected later in the season, but losses were not as severe. Phytophthora blight (*Phytophthora capsici*) continued to plague northern pepper fields. Sunscald and blossom end rot were prevalent in many non-irrigated peper fields.

Other Vegetables: Scab (*Streptomyces scabies*) was diagnosed on potatoes. Hollow heart, a noninfectious, high temperature related malady was diagnosed frequently on potatoes from homeowners' gardens. Rust (*Puccinia sorghi*) reduced the value of Indian corn. This disease causes premature death and blemishes on wrapper leaves. Common smut (*Ustilago maydis*) also was identified on sweet corn with the usual frequency. Rust (*Puccinia asparagi*) was identified on asparagus ferns from northern Indiana. Botrytis blast (*Botrytis cinerea*) and Alternaria purple blotch (*Alternaria porri*) were diagnosed on onions.

Agronomic Crops

Diseases—Corn: Seedling blight due to *Fusarium spp.*, primarily *Fusarium moniliforme*, was widespread. It was most pronounced and severe on young seedlings in fields where soil compaction, herbicide injury or other stress factors compounded the stresses of above normal temperatures and dry soil conditions. Most infection appeared to occur in the mesocotyl tissues. When infection occurred early in seedling development, many seedlings were killed. When infection occurred later as the nodal root systems were developing, seedlings were sometimes killed, but, more frequently, they were temporarily stunted and weakened. Consequently, many fields exhibited uneven plant development early in the season. In some fields increased Fusarium stalk rot at harvest time appeared to be related to the degree of seedling blight. Some genotypes were more affected than others.

Gray leaf spot (*Cercospora Zea-maydis*) was much more widespread in the southern half of Indiana in 1987 than it was in 1986, but individual fields appeared to be less severely affected (8). The disease was observed or reported from many of the counties south of Indianapolis. Races 1 and 2 of the Northern Corn Leaf Blight fungus (*Exserohilum turcicum*) were again identified from individual fields in 1987, but the disease was less damaging than in 1986. As in 1986, most of the Northern Corn Leaf Blight occurred in the east central and southern parts of the state. Southern Corn Leaf Blight (*Bipolaris maydis* race O), Northern Corn Leaf Spot (*Cochliobolus carbonum*) and Anthracnose leaf blight and top kill (*Colletotrichum graminicola*) were commonly found diseases, but caused little or no yield losses, except in some seed production fields. The leaf blight phase of Stewart's Disease (*Erwinia stewartii*) was widespread but damaging only in fields planted to highly susceptible inbreds and hybrids. The vector of this bacterial pathogen, the corn flea beetle, overwintered quite well due to the mild winter conditions, and high populations of the vector were present throughout the growing season.

Stalk rots were the most prevalent and damaging diseases to corn in 1987. Early maturity and good weather conditions at harvest time greatly reduced stalk rot losses in many fields. Premature killing of plants due to stalk rots was limited to individual fields, and reduced yield through light weight, chaffy kernels in addition to stalk lodging. Fusarium Stalk Rot (*Fusarium moniliforme*) and Anthracnose Stalk Rot (*Colletotrichum graminicola*) were the most prevalent stalk rots encountered. Gibberella Stalk Rot (*Gibberella zeae*) Diplodia Stalk Rot (*Stenocarpella maydis*) (*Diplodia maydis*), and Charcoal Stalk Rot (*Macrophomina phaseolina*) were other stalk rot diseases that were observed or reported, but occurred with less frequency than Fusarium and Anthracnose stalk rots.

Diplodia Ear Rot (*S. maydis*) was very severe in limited areas of southern Indiana. Some fields had 30% of the ears entirely rotted. While the disease was primarily limited to southern Indiana, light infections were found as far north as Tippecanoe County. Overall, Diplodia Ear Rot was more severe and widespread in Indiana in 1987 than it has been in more than 25 years (1,2,3,4,5,6,7,8).

Sorghum Downy Mildew (*Peronosclerospora sorghi*) was found for the second year in succession in Tippecanoe County. In 1987, the disease was identified from dent and pop corn as well as sorghum and Sudax.

Diseases—Soybeans: Most soybeans were planted in relatively dry, warm soils and there was a minimal amount of seedling blight problems observed or reported. Unlike corn, these soil conditions did not appear to stress the young soybean seedlings. Rhizoctonia Root Rot (*Rhizoctonia solani*) was a commonly reported early season problem, but in most cases this disease was found in association with suspected herbicide injury. The Phytophthora Root Rot organism (*Phytophthora megasperma* var *sojae*) was isolated from many specimens from areas that received heavy rainfall from sporadic thunderstorms

in July. In many cases, the organism was isolated from plants that did not exhibit typical Phytophthora Root Rot symptoms. *Verticillium* sp. was also isolated from several wilting soybean plants.

Foliar diseases such as Brown Spot (*Septoria glycines*) and Bacterial Blight (*Pseudomonas syringae* pv *glycinea*) were commonly found, but infection was light. Downy Mildew (*Peronospora manschurica*) was prevalent in east central Indiana, and especially in Henry and Randolph Counties. Yield losses from Downy Mildew were estimated to be minimal even in areas where the disease was widespread. Minor instances of Stem Canker (*Diaporthe phaseolorum*) were reported from individiual fields, primarily from the southern part of the state.

Sudden Death Syndrome, a disorder of unknown etiology, was more severe in southwestern Indiana in 1987 than it was in 1985 or 1986. Yield losses in individual fields were as much as 40 to 60%. The problem was identified for the first time in Putnam and Benton Counties, but yield losses were minor. Brown Stem Rot (*Phialophora gregata*) was observed or reported from several fields in the northwestern part of the state. Yield reductions in some fields affected with Brown Stem Rot were reported to be 10 to 20%. Charcoal Root Rot (*Macrophomina phaseolina*) was observed or reported from numerous fields in the state. The disease appeared to be most prevalent in the southern half of Indiana, although it was identified in several northern Indiana locations. Yield reductions of 20% or more were reported from severely infected fields.

Diseases—Wheat: Infectious disease were of minor importance in wheat production during 1987. Early in the spring, Septoria Leaf Blotch (*Mycosphaerella graminicola*; (*Leptosphaeria nodorum*), Powdery Mildew (*Erysipha gamminis* f. sp. *tritici*) and Tan Spot (*Pyrenophora trichostoma*) were present in many fields in the state. Dry weather, however, prevented these diseases from developing to damaging proportoins. Barley Yellow Dwarf Virus was probably the most widespread, but most infectionsoccurred in the spring and yield losses were not sizeable. Barley Yellow Dwarf Disease was very extensive and damaging to susceptible oat varieties.

Pythium Root Rot (*Pythium* spp.) was identified from two different fields. This is a rarely identified disease in this state, possibly due to confusion with other root pathogens. While the disease was identified from only two fields, the disease may have been more widespread.

Take-all (*Gaeumannomyces, graminia* var. *tritici*), Fusarium Root Rot (*Fusarium* spp.) and Rhizoctonia Root Rot and Sharp Eyespot (*Rhizoctonia solani*) were root rots also identified, but these disease were of minor importance, except in a few fields.

Diseases—Forages: Sclerotinia Crown and Stem Rot of alfalfa (*Sclerotinia trifoliorum; S. sclerotiorum*) caused severe damage to young plants in some fields that were seeded in the fall 1986. The disease was identified from fields scattered over the entire state.

Foliar and stem diseases of alfalfa were common throughout the state. The severity of these diseases was dependent upon the amount of rainfall received in a particular location. Lepto Leaf Spot (*Leptosphaerulina medicaginis*) and Spring Blackstem (*Phoma medicaginis*) were the most commonly observed. Both quality and yield were reduced in some fields. Anthracnost (*Colletotrichum trifoli*) occurred in some fields, but the disease appeared to be relatively light. However, anthracnose crown rot was suspected in some fields that suffered stand reductions during the summer.

Phytophthora Root Rot (*Phytophthora megasperma*) was identified in a few fields, but was not sever in 1987. The Crown Root Rot Complex, a disease of the crowns that can be induced by numerous fungi, appeared to cause stand reductions in many fields scattered over the state. Violet root rot (Rhizoctonia crocorum) was diagnosed on alfalfa from Knox County.

A leafspot on Fescue from northern Indiana was identified as *Stagnospora* sp.

Turfgrasses

Snow Molds (*Typhula* ssp.; *Fusarium* spp.) were observed in many areas in the late winter. Helminthosporium leafspot diseases (*Dreschlera* ssp.; *Bipolaris* spp.) were common throughout the state in the early spring on bluegrasses, fescues and ryegrasses. Leaf Rust (*Puccinia* spp.) occurred in many northern Indiana lawns late in the fall.

Patch diseases (*Leptosphaeria korrae*; *Phialophora graminicola*; *Rhizoctonia solani*) were common summer diseases identified on *Poa pratensis* and *Poa annua*. Due to the extreme environmental stresses placed on turfgrasses during the summer, it was difficult to ascertain whether turfgrass death was the result of disease or environmental conditions without laboratory studies.

Discussion

A compilation of plant disease information, such as this, could be an invaluable tool in determining the problems that require attention by extension personnel. Through proper diagnosis of plant problems and the subsequent rendering of proper methods of control it is hoped that both the economic and aesthetic losses caused by plant diseases can be reduced in future years.

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CELL BIOLOGY

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ABSTRACTS

The Effects of a Fish Oil Diet on Rat Lipoproteins. Daniel Belcher, William Stillwell, Stephen Wassall and Carol Langsford, Department of Biology and Physics, Indiana University-Purdue University at Indianapolis, Indianapolis, Indiana 46223.—Rats were fed identical diets supplemented with hydrogenated coconut oil (HCO) or with menhaden oil (MO), a fish oil rich in N-3 long chain, polyunsaturated fatty acids. High density lipoproteins (HDL) and low density liroproteins (LDL) were isolated from plasma, and fluidity and order were determined as a function of fatty acyl chain position in the lipoprotein monolayer, by fluorescence polarization and electron spin resonance (ESR). The HDL study showed a large difference in monolayer fluidity in the center region of the fatty acyl chains with the MO diet HDL being substantially less fluid than the HCO diet HDL. A parallel study with LDL indicated a small difference in monolayer fluidity in the center of the chains with the MO diet LDL being less fluid than the HCO diet LDL. These results were futher substantiated by molecular modeling.

The Production of Accessory Limbs in Axolotls. Margaret W. Egar, Department of Anatomy, Indiana University School of Medicine, Indianapolis, Indiana 46223.—Accessory limbs may be the result of several types of trauma in the salamander. The stimulus of implants of various kinds are all made more successful if a large nerve is deviated to the wound site. In the present experiments, a high yield of ectopic limbs was sought with nerve deviation alone. Success was achieved when a 2 mm patch of skin was removed at the proximal site of the deviated nerve. However, not all resulting growths continued development.

Microscopic examination (light, TEM and SEM) of the early blastemata revealed an accumulation of mesenchymal type cells that was continuous with the deviated nerve. The accessory blastema differed from that which forms at an amputation stump in having a central core of axons with capillaries and debris intermingled. This work was supported by a grant from the American Cancer Society, Ohio Division, Cuyahoga County Unit.

Fine Structure of the Labium of *Cenocorixa bifada* in Relation to Ionic Transport. Mohinder S. Jarial, Center for Medical Education, Ball State University, Muncie, Indiana 47306.—The labium of water boatman *Cenocorixa bifada* was studied by light, scanning and transmission electron miscrosopy. The triangular labium dorsally bear a deep groove flanked by a series of cuticular plates. Each plate bears two rows of crescent shaped depressions. The cuticular depressions in one row are placed in between those of the other row. The epithelium underlying the cuticle consists of narrow, elongate cells. A sinus containing particulate material exists between the cuticle and the epithelium. The

basal plasma membrane of the epithelial cells exhibits extensive infoldings that are closely associated with large mitochondria. These infoldings on the cytoplasmic side are covered by a particulate coat. The epithelial cells have centrally placed nuclei and their cytoplasm is rich in ER, mitochondria, vesicles and dense bodies. Their apical membranes are thrown into delicate invaginations. The large intercellular spaces contain vesicles and electron dense material. The labium takes up silver ions from dilute silver nitrate solution and becomes darkly stained. On the basis of these observations, the labium of *Cenocorixa bifada* is worthy of consideration as the site of ionic transport.

Kinetic Assay for Primary Cytotoxic T-Lymphocyte Clones. LAURA J. JENSKI, Department of Biology, Indiana University-Purdue University at Indianapolis, Indianapolis, Indiana 46223 and Beth Miller, Children's Hospital Research Foundation, Cincinnati, Ohio 45229.—Cytotoxic T-lymphocytes (CTL) aid in our defense against disease by killing abnormal cells such as virus-infected and cancer cells (targets). CTL and targets interact with an affinity amenable to analysis with methods designed for enzymes. We have developed a kinetic assay for primary murine CTL clones generated *in vitro*. We have shown that several assumptions underlying the Michaelis-Menten equation can be validated experimentally, and that there appears to be adequate justification for the use of "K_m" to estimate affinity. In normal naive spleen cell populations, we have found a broad range of affinities expressed by CTL clones responsive to foreign histocompatibility antigens. Selection or expansion of CTL clones with high target affinity, illustrated by a predominance of clones with small "K_m", may in some instances play an important role in generation of protection immunity.

Effect of Pentoxifylline on ESR Measured RBC Fluidity. F.W. KLEINHANS, D.D. NGUYEN AND S.T. BAREFOOT, Department of Medical Research, Methodist Hospital, Inc. of Indianapolis, Indianapolis, Indiana 46202 and Department of Physics, Indiana University-Purdue University at Indianapolis, Indianapolis, Indiana 46223.—Pentoxifylline (Trental) is a hemorheologic agent useful for the treatment of patients suffering from intermittent claudication and is believed to work in part by increasing the flexibility of erythrocytes. Previous *in vitro* studies in which red blood cells were forced through capillary tubes demonstrated an increased flow rate after treatment of the RBC with Trental. Our purpose in the electron spin resonance experiments was to duplicate the previous *in vitro* treatment protocols and determine whether there were any detectable membrane modifications using ESR spin label techniques. 5 doxyl steric acid was used which is sensitive to modifications of the lipid regions of the RBC membrane. Cells were held in Hepes buffer (+8 mM Ca++) and treated with 10 to 100 μg/ml of Trental for 30 to 150 min. No differences ($\Delta S > 0.007$) were seen in the 5DS order parameter, S. These results suggest that Trental may act on some other component of the RBCs such as the structural proteins associated with the lipid bilayer.

The Developmental Toxicity of Nicotine on the Planula of the Marine Hydrozoan, *Halocordyl disticha*. KATHLEEN J.S. KOLBERG AND VICKI J. MARTIN. Department of Biological Sciences, University of Notre Dame, Notre Dame, Indiana 46556.—Nicotine concentrates around the embryo and fetus in mammals and its developmental toxicity is of interest. This study examines the effects of nicotine on developmental processes in a simple invertebrate system, hydrozoan embryos. Nicotine administered at gastrulation and after development of the first nerve cells caused several developmental changes: 1) alteration in the ratios of derivatives of interstitial stem cells, 2) reversal of photactic behavior, 3) premature accelerated metamorphosis, 4) the polyps formed by the early, rapid metamorphosis showed several morphological abnormalities: irregular tentacle spacing, stunted tentacles and wrinkled head surface. The last two effects were mimicked

by exposing mature control planulae to a low dose of exogenous norepinephrine. Planulae tested with sucrose-phosphate-glyoxylic acid exhibited a fluorescence indicative of catecholamines. Treatment of planulae with several neuroactive drugs substantiated the presence of endogenous catecholamines. The effect of nicotine on metamorphosis could be mediated by the release of endogenous catecholamines. This study was funded by research grants from the Indiana Academy of Science.

The Effects of Vitamin C on Histone Acetylation and Phosphorylation in Mouse Fibroblasts. KYOUNGSOOK PARK AND DAVID A. PRENTICE, Department of Life Sciences, Indiana State University, Terre Haute, Indiana 47809.—Antioxidants such as vitamin C (L-ascorbic acid, AA) show protective effects on cellular aging and enhance cell growth *in vitro*, but the mechanism of action is unknown. One mechanism may be alteration of gene expression. Because changes in chromatin structure via histone modifications can affect gene expression, mouse fibroblasts (L cells) grown in serum-free medium were treated with AA to investigate its effect on histone modifications. AA (50 μg/mL) did not affect L cell growth. Cells were labeled with [^{3}H] lysine or [^{14}C] lysine to label histone proteins and then pulse labeled with [^{32}P] phosphate or [^{3}H] acetate to detect phosphorylation or acetylation, respectively. Extracted histones were resolved by SDS-PAGE, gel lanes sliced and labels detected by double-label liquid scintillation counting. Phosphorylation of histones H1 and H2A decreased in AA-treated cells, while acetylation of histones H3 and H4, as well as that of H2A and H2B, increased. Based on these results, one of the effects of AA may be alteration of histone modifications such as phosphorylation and acetylation thereby altering chromatin structure and gene expression.

Effect of Dietary Stearate and Linoleate on Prostaglandin Concentrations of Plasma in Normal and Tumor-bearing Strain A/ST Female Mice. TIMOTHY A. TRUE AND ALICE S. BENNETT. Department of Biology, Ball State University, Muncie, Indiana 47306.—Prostaglandins (PGs), more specifically PGE2, have been implicated in the evolution of breast cancer and may indicate the metastatic potential of that cancer. Dietary linoleic acid (18:2), a precursor of PGs, promotes the formation of mammary tumors whereas stearic acid (18:0) decreases tumorigenesis. The mechanisms involved in the promoter or inhibitor action are not known. In this study the effect of dietary 18:0 and 18:2 on PG concentrations of plasma removed from normal and mammary tumor-bearing female Strain A/ST mice was examined. Four high fat (15%) and two low fat (5%) diets containing varying percentages of 18:0 or 18:2 were used in this study. PGE2 concentrations were determined by HPLC analysis of their panacyl PG ester derivates, using a fluorescent detector. Variations of PG levels in plasma from mice fed experimental diets will be presented. Supported in part by a Sigma Xi Grant.

Phytohemagglutinin Binds to the Same Sites in the Ciliate *Stentor* as Does Con A. PATRICIA R. WALSH AND MICHAEL S. MALONEY, Department of Biological Sciences, Butler University, Indianapolis, Indiana 46208.—The plant lectins Con A and PHA are known to delay oral regeneration in the ciliate *Stentor coeruleus* and Con A appears to do so by binding to ciliary membranes only. Studies were initiated to determine whether PHA, which has effects identical to Con A, binds to the same sites on the cell surface. Cells were fixed and then exposed to 100 μg/ml of FITC-PHA for 30 min. Observations of these cells by fluorescence microscopy showed that PHA bound to the membranellar and body cilia exactly as Con A did. Furthermore, when regenerating cells were treated in the same manner, FITC-PHA also bound to the developing oral primordium as did Con A. Despite the similar binding patterns of the two lectins, α-methyl mannoside could not reverse the binding of FITC-PHA. Supported by NSF and a Penta Corporation Grant of the Research Corporation.

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An Optimized Genetic Code

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Introduction

In living organisms 20 amino acids are encoded by 64 codons. These numbers are often regarded as pure coincidences determined at random in some remote epoch when the genetic machinery of the cell was finalized [see e.g. refs 1 and 2]. However, it is not excluded that a deterministic mechanism is working and that, for instance, the number of coded amino acids is determined by the genetic code itself or its environment and therefore a systems theoretical approach may be appropriate.

The first investigator to consider the latter possibility in some detail seems to have been Garnow [3]. He derived the magic number 20 from a special model of the genetic apparatus. This particular model was later found to be less satisfactory and Garnow's derivation is therefore most likely not acceptable today.

A recent calculation of the correct number of the coded amino acids has been given by Soto and Toka [4]. This theory is based on a principle that defines the optimal code as one that minimizes for a given amount of information encoded, the product of the number of physical devices used by the average complexity of each device. The theory obtains the number 20, but does not give an explanation of the degeneracy and partitioning of the codons, which play such an important part in the genetic code problem.

In this work we present a derivation of the numbers involved in the genetic code problem that is based on the degeneracy of the codons and an assumption that the cell functions under the pressure of delivering an effective product. It is found that not only can the number of amino acids be derived in a simple manner but also the actual partitioning of degeneracy of the codons can be clarified.

Theory

The basic assumption of the theory is that the genetic code machinery of a cell is functioning under the constraint of optimizing its operations. Empirical genetic code data must be used as a guide to find the proper function to optimize.

Table 1 gives the genetic code in the so called universal or standard form (SGC) with the usual abbreviations for basis and amino acids [see e.g. ref 5]. It should be noted that the codons are divided into partitions according to the degeneracy of the codons

Table 1. The standard genetic code (see e.g. ref 5)

Leu UUA	Ser UCU	Gly GGU	Pro CCU	Tyr UAU	Ter UAA
UUG	UCC	GGC	CCC	UAC	UAG
CUU	UCA	GGA	CCA	Glu GAA	
CUC	UCG	GGG	CCG	GAG	Ile AUU
CUA	AGU	Ala GCU		Cys UGU	AUC
CUG	AGC	GCC	Asn AAu	UGC	AUA
Arg CGU		GCA	AAC	His CAU	
CGC	Thr ACU	GCG	Lys AAA	CAC	Met AUG
CGA	ACC	Val GUU	AAG	Aln CAA	Trp UGG
CGG	ACA	GUC	Asp GAU	CAG	Ter UGA
AGA	ACG	GUA	GAC	Phe UUU	
AGG		GUG		UUC	

with each partitioning corresponding to an amino acid or a terminator. Thus, for instance, the partition block for the amino acid Ser consists of six codons. This will be referred to as a 6-degeneracy. The various degeneracies are shown in Table 1.

The theory will be discussed in terms of a set of variables, $x_1, x_2, \ldots x_i, \ldots x_n$, where x_i gives the number of partitions of a particular degeneracy i. If there are a total of N possible codons we have the general constraint

$$\sum_{i=1}^{n} i\, x_i = N \qquad (1)$$

with n different partitionings.

In the case of the SGC, the number N is 64 since there are 64 codons generated from the alphabet of the four letters U, C, A and G arranged in groups of three. Here as usual U,C,A and G represent the four bases uracil, cytosine, adenine and guanine.

The standard genetic code as displayed in Table 1 shows clearly that partitions with an even number of degeneracies are favored in nature. In fact only three partitions occur for i = 1 and one partition with i = 3. This rarity of partitions with odd numbers is even more pronounced in some of the genetic codes that have been discovered recently to occur in mitochondria [see e.g. ref 6].

The predominance of partitions with an even number of degeneracies is most likely due to the physical character of the molecules involved. Thus if the purpose is to consider the genetic code from a systems point of view it seems reasonable to accept as a starting point the basic empirical fact of predominance of partitionings of even degeneracy. This argument is also supported by symmetry considerations using group theoretical calculations [7].

An inspection of the date in Table 1 shows that $2x_2(=20)$ is equal to $4x_4(=20)$ which in turn is approximately equal to $6x_6(=18)$. This suggests that the function to optimize should be a simple product function.

The simplest function that is reasonable to apply is therefore a multilinear function, C, given by

$$C(x_1 \ldots x_n) = k \prod_i x_i \qquad (2)$$

with k being a constant. The problem is then to optimize the function $C(x_1 \ldots x_n)$ with the contraint of eq (1). By using the Lagrange multiplier method with constant β the problem can be formulated as an optimization of a function F given by

$$F = k \prod_i x_i - \beta \sum i x_i \qquad (3)$$

From the conditions $\partial F/\partial x_i = 0$ (i = 1,...n) we therefore obtain the following system of equations:

$$\begin{aligned} &k \prod_{i \neq m} x_i = \beta m \qquad (m = 1 \ldots n) \\ &\sum i\, x_i = N \end{aligned} \qquad (4)$$

to determine the n parameters x_i

Results and Discussion

Before solving the system (4) it is convenient to introduce the approximation stated in the previous section consisting of neglecting terms with odd degeneracy. We also use the empirical fact that there are no known eight-fold degeneracies. Thus specializing to the case N = 64, and i = 2, 4 and 6 the system of equations (4) reads:

$$\begin{aligned} k\, x_4\, x_6 &= 2\beta \\ k\, x_2\, x_6 &= 4\beta \\ k\, x_2\, x_4 &= 6\beta \\ x_2 + 2x_4 + 3x_6 &= 32 \end{aligned} \qquad (5)$$

This system has the following set of solutions:

$$\begin{aligned} x_2 &= 10.68 \\ x_4 &= 5.34 \\ x_6 &= 3.56 \end{aligned} \qquad (6)$$

This corresponds to 11 partitions with 2-degeneracy, 5 partitions with 4-degeneracy and 4 partitions with 6-degeneracy. The total number of partitions is 20.

An examination of the SGC as displayed in Table 1, shows that the code has 10 partitions with 2-degreneracy, 5 partitions with 4-degeneracy and 3 partitions with 6-degenercy. There is also 1 partition with 3-degeneracy and 3 partitions with 1-degeneracy.

The theoretical model is therefore in good agreement with the SGC partitions. This is especially true if the originally calculated numbers i.e. $x_2 = 10.68$, $x_4 = 5.34$ and $x_6 = 3.56$ are taken into account in the comparison and not just the rounded off, integer numbers.

One can also compare the prediction of the model with the genetic code as expressed in mitochondria of some species having a genetic code differing from SGC. Thus, for instance, in Protozoa [6] the genetic code has changed somewhat from SGC in that the codon UGA codes for the amino acid trp and is not a terminator. This changes the pattern of partitions into 11, 5 and 3 partitions for 2-,4- and 6-degeneracy respectively, which is in excellent agreement with the model values.

Table 2 gives a summary of experimental values and the predictions of the model.

Table 2. Number of degeneracies in SGC, some mitochondrial genetic codes and the corresponding model values (numbers in parantheses are the actual calculated values). Mitochondrial data are from Breitenberger [6]

Object	1-degen.	2-degen.	3-degen.	4-degen.	6-degen.
SGC	3	10	1	5	3
Mammals	0	14	0	6	2
S. cerevisiae	1	11	1	5	3
N. crassa	1	11	1	5	3
Protozoa	1	11	1	5	3
Model	—	11(10.68)	—	5(5.34)	4(3.56)

The general trend indicated by the data is in good agreement with the model. Other expressions than the one given by relation (2) have been tried. However, the tabulated relation seems to be the optimum one.

Conclusion

Most biological structures are determined by physical as well as system contraints. In the present work it is assumed that the physical constraint on the genetic code determines the basic 2-degeneracy. However, this degeneracy is modified by system constraints in such a way that some higher degeneracy is favored. The simple function that has been used seems to explain the gross structure of the existing partitions. The exact biological interpretation of the function is left open at this time. This procedure is therefore similar in spirit to the group theoretical arguments given by Findley and Mc Glynn [7] in that a mathematical structure is assumed without first introducing a particular biological func-

tion. Future work on the model must obviously address the interpretation question as well as the question concerning the actual assignment of codons to a particular amino acid. Such a task will most likely require additional physical as well as evolutionary considerations.

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The Effects of Novel Growth Factors on T-Lymphocyte Proliferation

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Introduction

The major growth factor for T lymphocytes is interleukin 2 (IL2); however, a number of studies have implicated prolactin (PRL) and other lactogens (e.g., human growth hormone, hGH) as important immune regulators. Receptors for lactogens are present on lymphocytes (17,18,20) and lactogens have been shown to promote lymphocyte proliferation (20). PRL can restore immunocompetence in hormone-deficient animals (12,13), it induces the secretory immune system in the mammary gland (24) and decreased PRL binding/action may play a role in mediating the immunosuppressive actions of cyclosporin (8). Recently it has become possible to examine the immunostimulatory actions of PRL using a rat T-cell line, the Nb2 lymphoma (20,22). These cells proliferate in response to low concentrations of lactogens (i.e., PRL. hGH) and in response to the normal T-cell mitogen (IL2 (14).

Recent studies suggest that a number of novel growth factors may regulate T-cell function. Transforming growth factor-β (TGF-β), which is produced by a number of tissues including monocytes (1) and T lymphocytes (9), inhibits the activity of immune cells. In tonsillar T-lymphocytes, TGF-β inhibits IL2-stimulated proliferation and it inhibits induction of the IL2 receptor by concanavalin A (9). TGF-β also inhibits concanavalin A-stimulated growth of mouse thymocytes (15). Activation of T lymphocytes increases the synthesis and secretion of TGF-β and up-regulates the receptor for TGF-β (9). TGF-β has been shown to decrease the cytolytic activity of human natural killer cells and to decrease the sensitivity of these cells to α-interferon (16). TGF-β also stimulates the expression of the protooncogene c-*sis* (11), a molecule which codes for one subunit of platelet derived growth factor (PDGF), and it has been proposed that PDGF may mediate some actions of TGF-β (11). In this regard, PDGF has been shown to decrease natural killer cell activity (6). Another novel factor that regulates cells of the immune system is tumor necrosis factor (TNF). It has been shown recently that TNF inhibits proliferation of HL-60 cells (10), a human lymphocyte cell line. Like TGF-β, TNF is produced by mononuclear phagocytes (4).

In this study, we have used Nb2 cells to examine the effects of these novel growth factors on lactogen-stimulated T-cell growth. The data show that TGF-β, TNF and PDGF inhibit proliferation of Nb2 cells in response to lactogens. We also examined the actions of fibroblast growth factor FGF), but this factor had no effect on lactogen-stimulated Nb2 cell growth. Overall, these data further support immunosuppresive roles for TGF-β, TNF and PDGF.

Methods and Materials

Materials—hGH (79-7-23H) and TGF-β were gifts of Dr. H.G. Friesen and Dr. M.B. Sporn, respectively. Rat IL2 and PDGF were obtained from Collaborative Research, Inc. (Lexington, MA), FGF (basic) was purchased from R&D Systems, Inc. (Minneapolis, MN) and TNF was donated by Genentech, Inc. (South San Francisco, CA). Ovine PRL (oPRL) was obtained from the Hormone Distribution Program, NIH.

Cell culture and quantitation—The Nb2 cells were grown in Fischer's medium supplemented with 10% fetal bovine serum, 10% horse serum, 1 mM 2-mercaptoethanol, 100 U/mL penicillin and 100 μg/mL streptomycin, and were maintained in a humidified atmosphere of 5% CO_2/95% air at 37 °C. Cell proliferation was quantified according to the bioassay of Tanaka *et al.* (22). Approximately 24 h prior to the addition of test substances, the Nb2 cells were growth-arrested by removing fetal bovine serum from the medium (i.e., resuspended in "assay medium"). Cells were exposed to test substances for 3 days except where indicated. Cell number was determined using a Coulter model ZM counter (Coulter Electronics, Inc., Hialeah, FL).

Results and Discussion

As shown in earlier studies (14,20,22) the Nb2 cells proliferated in response to the lactogen hGH and in response to IL2 (Fig. 1). Proliferation in response to both mitogens

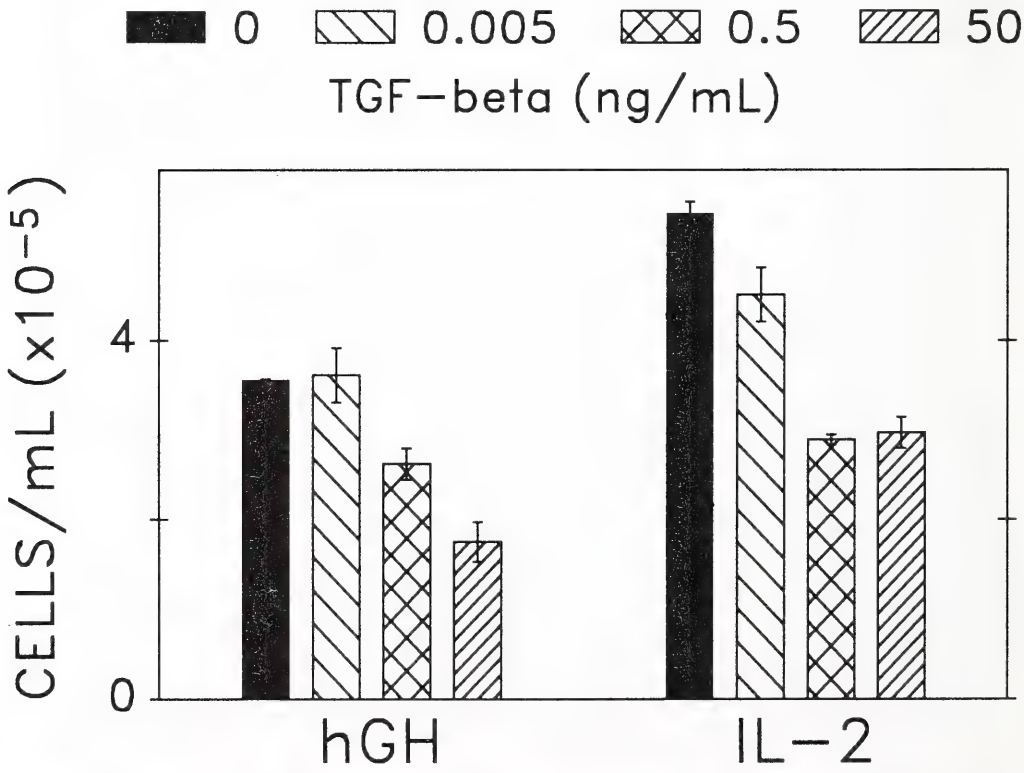

FIGURE 1. Inhibitory effects of TGF-β on mitogen-stimulated proliferation of Nb2 cells. Cells were plated in assay medium at a concentration of 1 x 10^5 cells/mL in the wells of 24-well culture dishes. The cells were exposed to hGH (1 ng/mL) or rat IL2 (20 half-maximal units/mL) ± TGF-β (concentrations indicated) for three days.

was inhibited by low concentrations of TGF-β, but proliferation could not be totally blocked even by high concentrations of TGF-β. Preliminary data suggest that this incomplete inhibition of Nb2 cell growth stems from the fact that the inhibitory actions of TGF-β are not expressed in the first round of cell division but only in the second or subsequent rounds (data not shown). This inconsistent timing in the expression of the actions of TGF-β led to considerable variability in the level of inhibition detected in the 3-day assays; however, the results of 15 studies with TGF-β indicated that this factor is a potent immunosuppressive agent.

PDGF also inhibited the proliferation of Nb2 cells in response to the lactogen PRL (Fig. 2). As observed with TGF-β, only partial suppression was obtained using a high

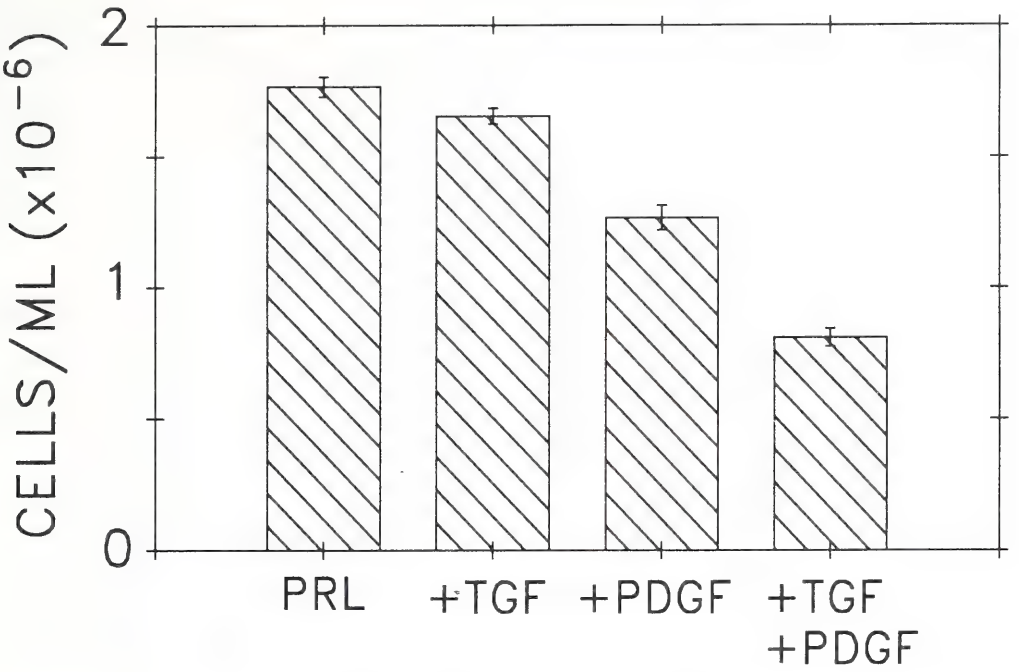

FIGURE 2. Inhibitory effect of PDGF on PRL-stimulated Nb2 cell growth. Cells were plated in assay medium at a concentration of 1 x 10^5 cells/mL in the wells of 96-well culture dishes. Cells were exposed to ovine PRL (5 ng/mL) ± PDGF (50 half-maximal units/mL) and/or TGF-β (5 ng/mL) for three days.

concentration of PDGF. When PDGF was combined with TGF-β, the inhibition of Nb2 cell proliferation appeared to be more than additive. However, the inhibition attained using TGF-β in these experiments was minimal; therefore, it is not possible to state at this time whether there is synergism between the two factors. Inasmuch as TGF-β stimulates c-*sis* expression, it is possible that a PDGF-like molecule contributes to the actions of TGF-β.

A preparation of TNF prepared by recombinant technologies exerted strong immunosuppressive actions in the Nb2 cell assay. At a concentration of 1 μg/mL, TNF totally blocked cell proliferation in response to PRL (Fig. 3). It is unknown, however, whether the high concentrations of TNF required for inhibition of Nb2 cell growth would be encountered under physiological conditions. If these concentrations can be generated locally, then TNF, or perhaps the closely related protein lymphotoxin (7), may be an important immunoregulatory factor.

FGF, an agent which has not been reported to affect immune function, did not alter Nb2 cell proliferation when used at concentrations comparable to those used in other proliferation studies (Fig. 4). Thus, our data suggest that FGF does not play a role in regulation of T-lymphocyte proliferation.

The mechanisms through which TGF-β, PDGF and TNF inhibit the actions of lactogens and IL2 are unknown at this time, however, all three of the immunosuppressive factors have been shown to stimulate prostaglandin synthesis (5,19,23). It is possible, therefore, that these factors inhibit lymphocyte proliferation by increasing prostaglandin levels. In this regard, it has been shown that prostaglandins inhibit the proliferation

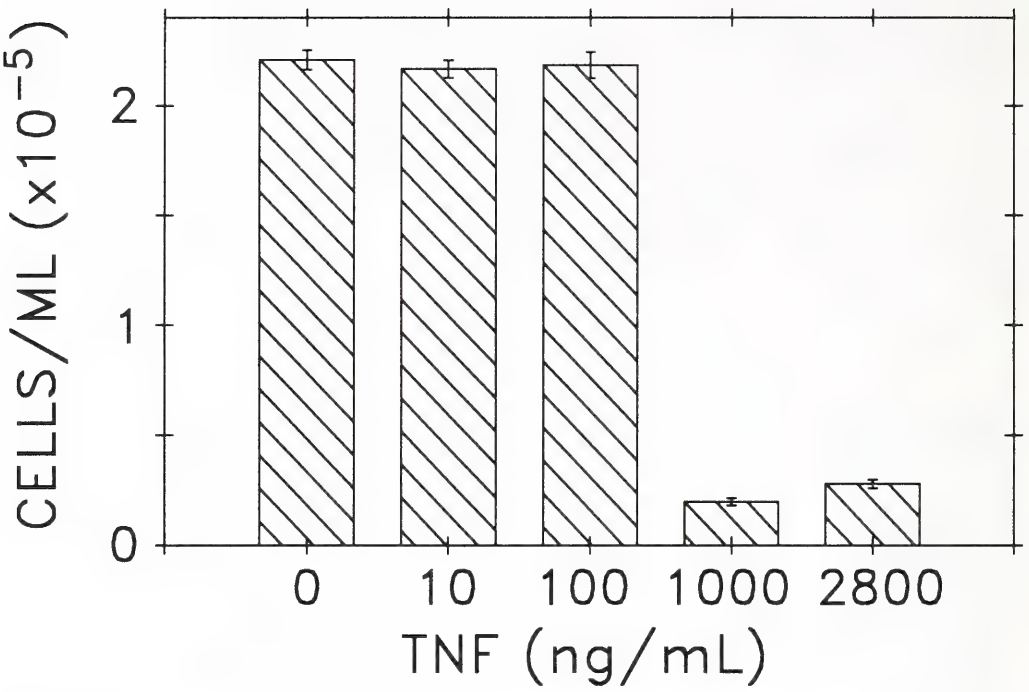

FIGURE 3. Inhibitory effect of TNF on PRL-stimulated Nb2 cell growth. Cells were plated in assay medium at a concentration of 2 x 10^4 cells/mL in the wells of 96-well culture dishes. Cells were exposed to ovine PRL (5 ng/mL) ± TNF (concentrations indicated) for five days.

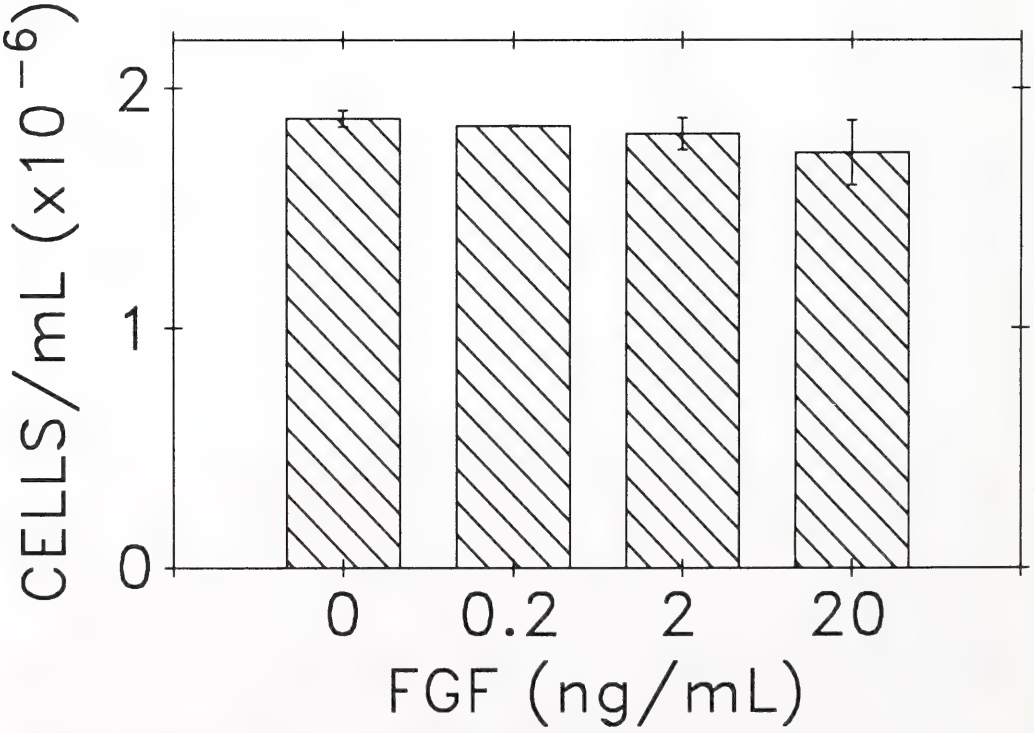

FIGURE 4. Effect of FGF on PRL-stimulated Nb2 cell growth. Cells were plated in assay medium at a concentration of 1 x 10^5 cells/mL in the wells of 96-well culture dishes. Cells were exposed to ovine PRL (5 ng/mL) ± FGF (concentrations indicated) for three days.

of T lymphocytes (2), perhaps through an increase in cAMP (3). Thus, our working hypothesis at this time is that TGF-β, PDGF and TNF regulate lymphocyte proliferation by increasing the production of prostaglandins.

Acknowledgments

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The Role of Ion Channels in Plant Protoplasts

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ABSTRACT

Plant hormones such as abscisic acid (ABA) regulate growth and a variety of physiological responses, but their mechanism of action is not well-defined. We employed patch voltage-clamp to study a voltage-dependent. TEA^+ sensitive K^+ channel in *Vicia faba* guard cell protoplasts and an ethacrynic acid and Zn^{++-} sensitive Cl^- channel in *Asclepias tuberosa* suspension cell protoplasts. Single channel K^+ conductance in *Vicia faba* guard cell protoplasts was 65 ± 5 pS and the mean open time was 1.25 ± 0.30 msec at 150 mV. While auxins and 2,4-D had no effect, micromolar levels of ABA increased the mean open time of *Vicia faba* K^+ channels and caused bursts of openings that were sufficient to account for ABA-dependent stomatal closure. In independent experiments *in vivo* measurements of stomatal aperature were used to demonstrate that ABA-dependent stomatal closure could be inhibited by EGTA or nifedipine. *Asclepias tuberosa* suspension cell derived protoplasts had K^+ and Cl^- channels with single channel conductances of 40 ± 5 pS and 100 ± 17 pS respectively. Mean mean open time for K^+ channels was 4.5 ± 0.4 msec at 100 mV, while Cl^- channels had a mean open time of 8.8 ± 1.2 msec and were blocked by Zn^{++} and ethacrynic acid. In contrast to *Vicia faba* K^+ channels, Cl^- channels in *Asclepias tuberosa* were blocked by micromolar levels of ABA, but again were not affected by auxins or 2,4-D.

Introduction

Plant hormones such as abscisic acid (ABA) regulate developmental events and physiological responses (5,7,12,15), but the factors linking hormonal signals and intracellular events are not well-defined. Ions cross cell membranes through quasi-permanent aqueous pores, referred to as ion channels, that may be gated by transmembrane electric field changes (voltage-dependent channels), or activated by one or more external stimuli (agonist-activated channels). In animal cells, hormones affect receptors coupled to ion channels, with subsequent ion fluxes or voltage shifts regulating intracellular second messengers. Binding proteins for ABA exist in various cells (1) and plant cells have been shown to contain cation and anion-selective channels by conventional voltage-clamp (2,3,11,13,26,30). There is also considerable recent evidence for Ca^{++}-dependent second messenger systems in a variety of plant tissues (1,5,14,18,19,20). Until recently the cell wall hindered direct studies of ion channels in plants, but patch-clamp of isolated protoplasts has made it possible to resolve single ion channels (16,27). *Vicia faba* guard cells were shown to have K^+ channels with a conductance of 20-30 pS (27), large enough to produce the net K^+ fluxes associated with ABA-dependent stomatal closure (15,28). Channels carrying maleate and K^+ were seen in vacuoles from barley leaf mesophyll protoplasts (9), while TEA^+-sensitive and TEA^+-insensitive K^+ channels with conductances of 100 pS and 30 pS respectively were observed in protoplasmic droplets from *Chara corallina* (11).

One important question is whether the ion channels observed in patch-clamped protoplasts are directly involved in significant physiological responses of plant tissues. To

resolve this, we measured the effect of ABA on stomatal aperature and guard cell protoplast diameter and tested the ability of the well-known Ca^{++} channel blocking agents EGTA and nifedipine to inhibit ABA-dependent changes in guard cell volume in *Vicia faba*. We then employed patch voltage-clamp to study two different, plant hormone-sensitive ion channels. The activity of a voltage-dependent K^+ channel in *Vicia faba* was enhanced by micromolar levels of ABA, whereas ABA blocked the Cl^- channel in *Asclepias tuberosa*. Demonstration of a qualitatively distinct hormone specificity suggests membrane-bound ion channels have a significant role in plant cell physiology.

Methods

Plant Protoplasts

Cell suspension culture-derived protoplasts were obtained from callus of *Asclepias tuberosa* (4) maintained on a rotary shaker in liquid Murashige-Skoog medium (17), with 5.0 mg/L adenine, 0.5 mg/L 2,4-dichlorophenoxyacetate (2,4-D), 0.5 mg/L benzyl adenine, 0.1 gm/L myoinositol, 0.4 gm/L casein hydrolysate, and 2% sucrose (pH 5.8, ref. 4) under fluorescent ceiling lights at 25 °C. To obtain protoplasts, 6-14 day old suspension cultures were first filtered through 400 um sterile gauze, and then centrifiged at 5000 rpm for 10 minutes. The pellet was then resuspended and digested for 4-12 hours at 25 °C (on a reciprocal shaker) in an equivolume (25 ml) solution of 0.5% Macerase and 1.0% Cellulysin (Calbiochem) in 350 mM mannitol, 350 mM sorbitol, 6 mM $CaCl_2$, 0.7 mM $NaHPO_4$ and 3 mM MES as pH = 5.8. Protoplasts were washed, filtered through sterile gauze (100 um and 40 um) and resuspended in 100 mM saline (NaCl, CsCl, or Cs^+ glutamate, 250 mM mannitol, 250 mM sorbitol, 2 mM $CaCl_2$, 0.7 mM $NaHPO_4$, pH 5.8).

Guard cell protoplasts were isolated from leaves of broad bean, *Vicia faba*. Leaflets from 3 week old plants were rinsed 3 times in a basal medium (BM) containing 550 mM sorbitol and 5 mM MES adjusted to pH 5.8 with KOH (33). All solutions were filter-sterilized using 0.22 um filters (Millipore). Leaflets were vacuum infiltrated for 3 minutes with 0.8% macerase (Calbiochem) and 1.0% dextran sulfate in BM and incubated in this solution at 25 °C for 20 minutes on a reciprocal shaker. Epidermal peels from the abaxial leaf surface were taken under a sterile hood and incubated in 0.5% macerase, 1.0% cellulysin (Calbiochem) and 0.5% dextran sulfate in BM for 12 hours on a reciprocal shaker (low speed) at 25 °C. To purify guard cells, the incubation medium was filtered through a 70 um nylon mesh to remove debris. Cells were centrifuged at 2000 rpm for 10 minutes in a clinical centrifuge, washed in BM and recentrifuged at 2000 rpm for 10 minutes in a clinical centrifuge, washed in BM and recentrifuged at 2000 rpm for 3 minutes twice. The last pellet was resuspended in a minimum amount of 100 mM NaCl, 300 mM sorbitol, 2 mM $CaCl_2$ and either 0.7 mM $NaHPO_4$ or 5 mM MES adjusted to pH 5.8. Although protoplasts from mesophyll and epidermal cells were released during digestion, few mesophyll protoplasts were present in the final suspension.

Stomatal Aperature

Stomatal aperature in *Vicia faba* was measured at 400× with a Nikon inverted phase-contrast microscope equipped with an ocular micrometer. Epidermal peels were obtained from the same leaves as used for protoplast isolation, with the peels subsequently floated in Ca^{++}-free distilled water in the dark for 30 minutes, and then exposed to light and CO_2-free, humidified air. Stomatal aperature was measured after 2-3 hours. Either ABA alone or ABA plus an inhibitor were then added to the bathing solution, with stomatal aperature redetermined 2-3 hours later. In each experiment 8-10 cells were measured, the data being expressed in terms of mean ± standard error. All differences noted were significant with $p < 0.01$ (Student's t-test).

Patch-Clamp Recording

Single channel recordings and whole-cell voltage-clamp were achieved using standard techniques (ref. 8, shown schematically in Figure 1). Patch pipettes were made us-

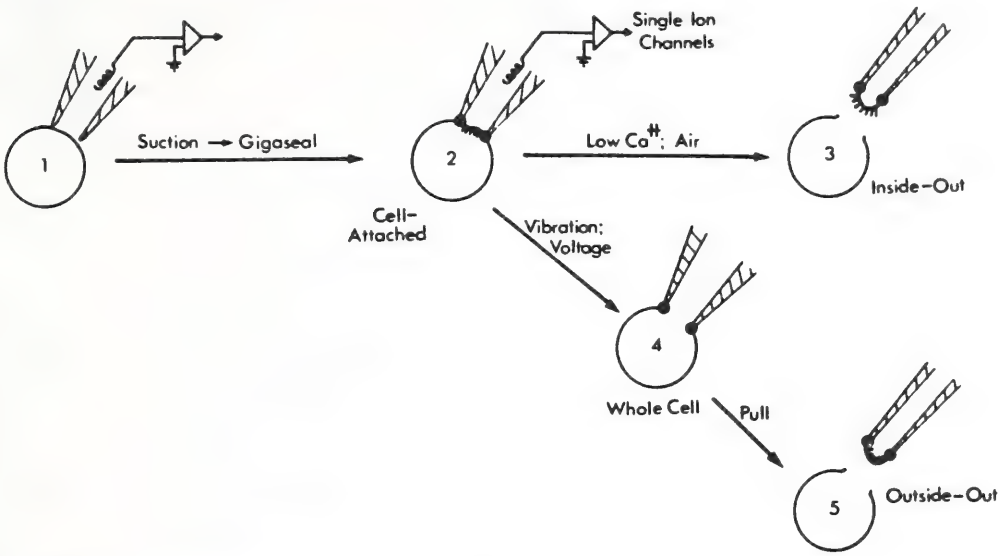


FIGURE 1. The principles of patch voltage-clamp.

ing a Kopf puller and had resistances of 8-12 Megohm when filled with the recording solution. Patch pipettes were gently brought into contact with protoplasts until resistance increased to 50-100 Megohm, with suction then applied to the pipette interior to promote high resistance seal (10-50 Gigohm) formation. Once a high resistance seal was formed, patch pipettes could be pulled away from the cell, giving excised patches in inside-out or outside-out conformations, depending on the Ca^{++} concentration.

The internal (pipette) solution was 100 mM KCl, CsCl, K^+ gluconate, or Na^+ gluconate buffered with 5 mM $NaHPO_4$ or Hepes to pH 5.80 ± 0.05. The external (bathing) solution was 100 mM NaCl, CsCl, Na^+ gluconate, or K^+ gluconate, again at pH 5.80 ± 0.05. Internal and external solutions contained 2 mM $CaCl_2$ unless otherwise noted and experiments were performed at room temperature of 22-25 °C. Currents were filtered at 3-10 KHz by a 4 pole Bessel filter, monitored with a List EPC-7 patch-clamp and sampled at 33 usec intervals via a Teckmar Labmaster board (Axon Instruments) by an IBM PC-XT, controlled using the program pCLAMP (Axon Instruments). Membrane potentials are given assuming that the protoplast exterior was a zero voltage. Note that in the whole-cell clamp and outside-out excised patch configurations positive pipette potentials correspond to membrane deplorizations, while in both the cell-attached and inside-out excised patch configurations positive pipette potentials correspond to hyperpolarizations.

Single-channel data was analyzed and plotted as amplitude and duration histograms, with conductance and mean open times determined by pCLAMP. Series resistance was 80-90% compensated and capacity currents subtracted by the analog circuitry in the EPC-7. Whole-cell currents were corrected for linear leakage current by a P/5 protocol. In this protocol currents were first measured for full-sized voltage steps (± P) in regions where current-voltage relations were nonlinear (deplorizations activated outward K^+ current and hyperpolarizations inward Cl^- currents), then measuring currents using voltage steps scaled to one-fifth the size (± P/5) using a holding, or reference potential in a range

where the current-voltage relation was linear and there was no apparent ion channel activation or inactivation. Reversal potentials were determined under biionic conditions using Tris as an impermeable cation substitute and gluconate as an impermeable anion, with the selectivity ratios estimated after correction for ion activity (10). in some experiments concentrated solutions of tetraethylammonium (TEA^+) and 4-aminopyridine (4-AP) were added to give a final concentration of approximately 10 mM, while abscisic acid (Sigma Chemical), Zn^{++} and ethacrynic acid (Sigma Chemical) were used at 10-100 uM. Some solutions were rendered Ca^{++} free using an EGTA buffer solution with $[Ca^{++}] = 10^-$ 8M, while nifedipine (Sigma Chemical) was used at 100 uM. All data are expressed as means ± standard errors for 5-8 independent experiments.

Results

Ion Channels in Vicia faba

In whole-cell patch-clamp *Vicia faba* guard cell protoplasts had voltage-dependent outward currents for depolarizations greater than 50-60 mV (Figure 2A). These currents were completely inhibited when 10 mM TEA^+ was added to the bathing solution and when Cs^+ was substituted for K^+ in the patch pipette. Time-dependent activation of outward current in *Vicia faba* was slow compared to outward membrane currents in nerve or muscle cells, with half-activation times of 400-450 msec at +80 mV, 200-225 msec at +100 mV, and 90-110 msec at +150 mV. In symmetrical 100 mM KCl solutions the instantaneous current-voltage relation for these currents was linear, reversing at a potential of 2 ± 4 mV. When the external solution was 100 mM NaCl and the pipette contained 100 mM KCl the reversal potential was −80 ± 4 mV. These data provide an estimated of 20:1 for the K^+/Na^+ selectivity ratio.

Figure 2B shows typical single channel K^+ currents for *Vicia faba* in an inside-out patch held at +100 mV before, during and after application of 10 uM ABA. Under control conditions there was one high-conductance channel and one or more channels of lower conductance that we were unable to resolve. Extrapolation of single-channel currents for the large channel yielded a reversal potential of −81 ± 7 mV when the pipette solution was 100 mM NaCl and the bath solution was 100 mM KCl, suggesting this was indeed a K^+-selective channel. Single channel K^+ conductance averaged 65 ± 5 pS in 7 patches and was voltage-independent. As for whole-cell currents, single K^+ channels were blocked by 10 mM TEA^+ (Figure 2B, traces 10-12) and could not be observed with Cs^+ in the patch pipette.

Exposure of *Vicia faba* guard cell protoplasts to 10 uM ABA increased the K^+ channel activity. Not only did K^+ channels open more frequently, but many channel openings occured in bursts lasting 50-100 msec (traces 6-10 in Figure 2B). ABA also increased mean open time within a burst. Under control conditions mean open time was 0.60 ± 0.15 msec at +80 mV. 0.80 ± 0.12 msec at + 100 mV, and 1.25 ± 0.18 msec at +150 mV. In the presence of ABA a satistical analysis restricted to well-separated openings yielded mean open times of 1.20 ± 0.24 msec at +80 mV and 2.05 ± 0.22 msec at +150 mV, all significantly longer than the control values. In contrast to ABA, the plant hormones kinetin (100 uM) and indole-3-acetic acid (IAA; 100 uM) had no effect on whole-cell or single-channel K^+ currents.

Ion Channels in Asclepias tuberosa

Two voltage-dependent conductances were observed in *Axclepias tuberosa* protoplasts. In 15-20% of the protoplasts examined depolarizations of 40-150 mV activated time-dependent outward membrane currents in symmetrical solutions containing 100 mM KCl (Figure 3), while hyperpolariztions had no effect. In symmetrical 100 mM KCl solutions the instantaneous current-voltage curve reversed at 5 ± 4 mV. When the external solution was 100 mM NaCl and the pipette contained 100 mM KCl the average reversal

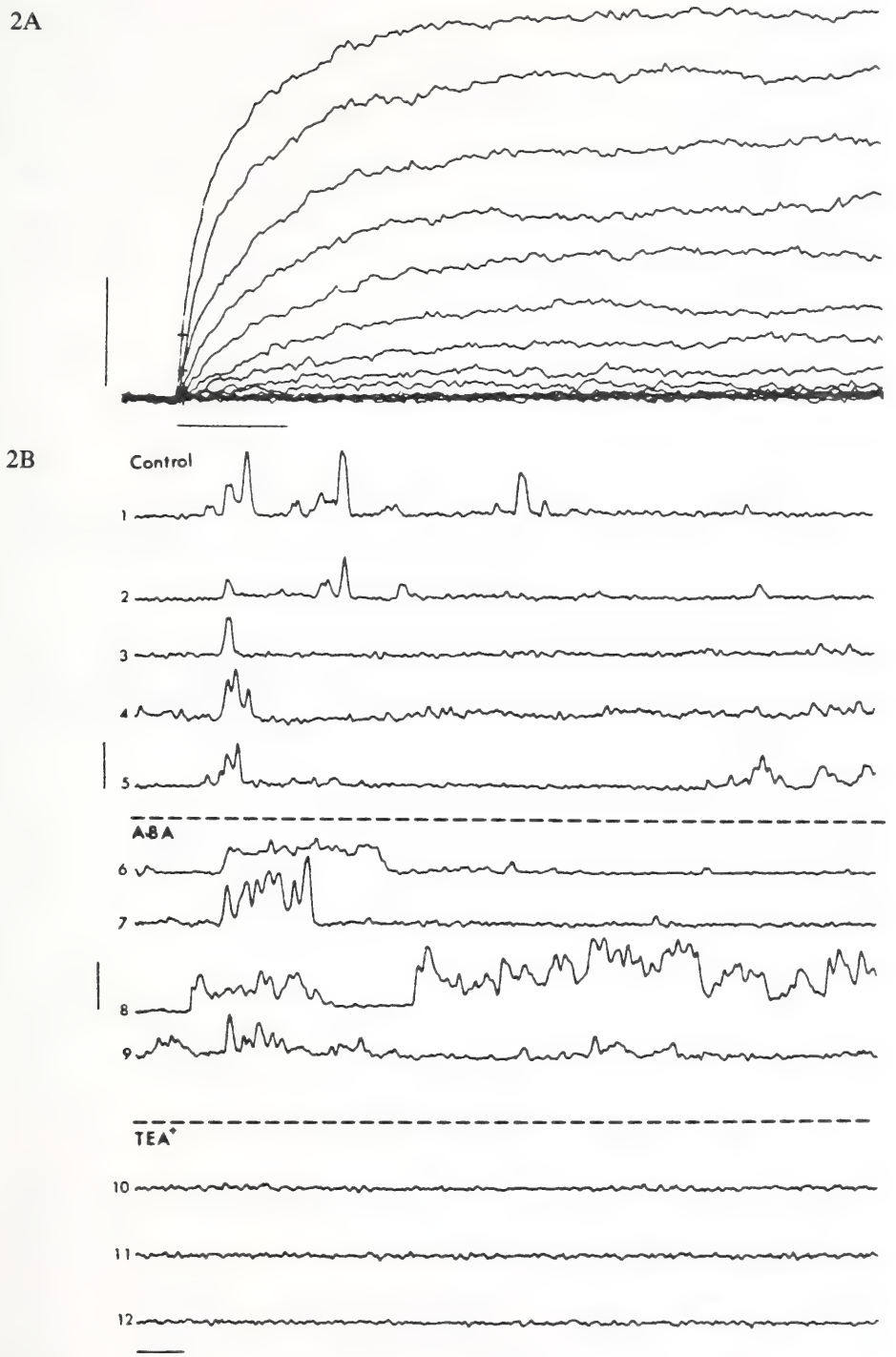


FIGURE 2. Part A shows whole-cell outward K^+ currents in *Vicia faba* guard cell protoplasts for depolarizations of 10-150 mV (10 mV increments). The pipette solution was 100 mM KCl, 2 mM $CaCl_2$ buffered with 5 mM Hepes to pH 5.8, while the bath contained 100 mM NaCl. Current and time calibrations are 100 pA and 500 msec respectively. Part B shows single channel K^+ currents in an inside-out patch held at a potential of + 100 mV relative to the grounded bathing solution before (control), during, and after (recovery) application of 10 uM ABA. Current and time calibrations are 5 pA and 10 msec respectively. Numbers refer to consecutive traces acquired in an event-triggered mode.

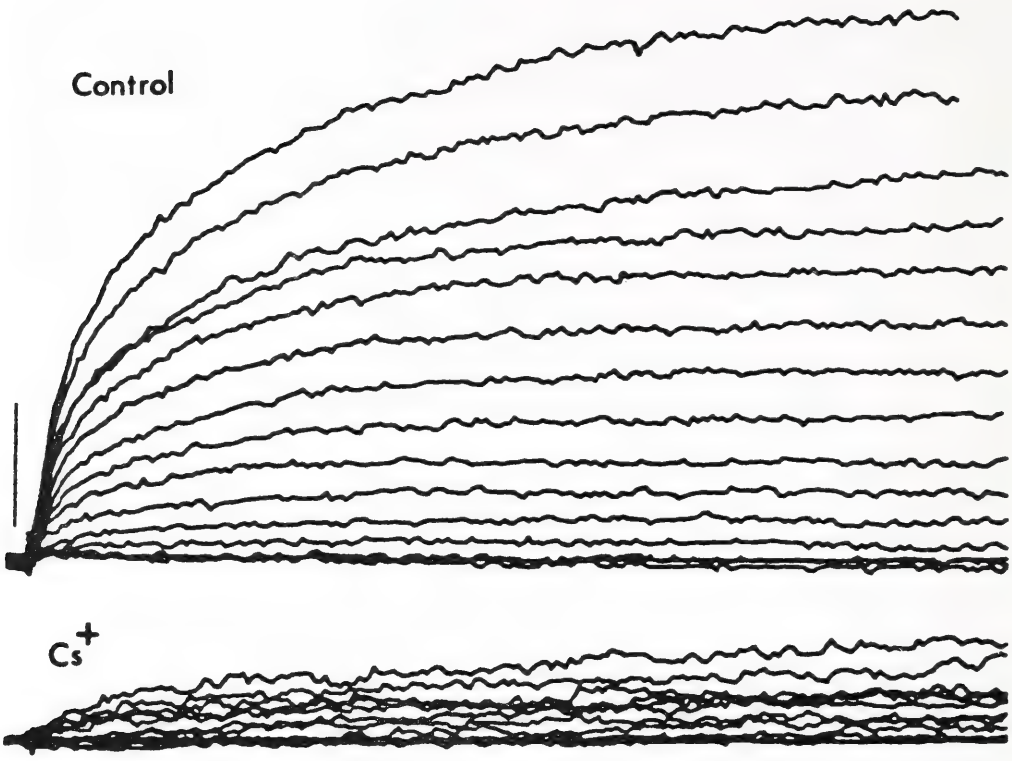


FIGURE 3. Whole-cell outward K^+ currents (upward deflections) in *Asclepias tuberosa* protoplasts for voltage steps of 10-150 mV (10 mV increments) before and after addition of sufficient Cs^+ to yield a final concentration of 10 mM. The pipette and both solutions were 100 mM KCl, 2 mM $CaCl_2$ buffered with 5 mM Hepes to pH 5.80 ± 0.05. Current and time calibrations are 100 pA and 10 msec respectively.

potential was −68 ± mV, whereas a value of 54 ± 6 mV was obtained in 100 mM KCl when the patch pipette contained 100 mM NaCl. The calculated K^+/Na^+ selectivity ratio for the *Asclepias tuberosa* K^+ channel was approximately 15:1. Half-activation times were 300-350 msec at +50 mV and 180-200 msec at +130 mV, also similar to the time course of K^+ currents in *Vicia faba* guard cell protoplasts. Outward currents were blocked by TEA^+ but, in contrast to *Vicia faba*, 100 uM ABA had no effect.

Figure 4A shows single channel K^+ currents in *Asclepias tuberosa* for an inside-out patch held at a potential of +100 mV. Extrapolation of single-channel currents yielded a reversal potential in symmetrical 100 mM KCl of 2 ± 5 mV, −72 ± 7 mV when the pipette (external) solution was 100 mM NaCl and the bathing (internal) solution was 100 mM KCl, and 56 ± 5 mV when the bath solution was 100 mM NaCl and the pipette contained 100 mM KCl. Selectivities derived from these data were the same as those obtained from whole-cell currents. As for whole-cell currents, single-channel K^+ currents were blocked by TEA^+, but were unaffected by ABA, IAA or 2,4-D. Single K^+ channel conductance averaged 40 ± 5 pS in 7 different patches, with a mean open time of 4.5 ± 0.5 msec.

Approximately 80% of *Asclepias tuberosa* protoplasts examined lacked time-dependent outward K^+ currents. Instead hyperpolarizations of 50-150 mV elicited a voltage-dependent inward currents in symmetrical 100 mM KCl or CsCl (Figure 5). In symmetrical 100 mM KCl or NaCl solutions, or with 100 mM KCl in the pipette and 100 mM NaCl in the bath, the reversal potential averaged −3 ± 6 mV. When gluconate

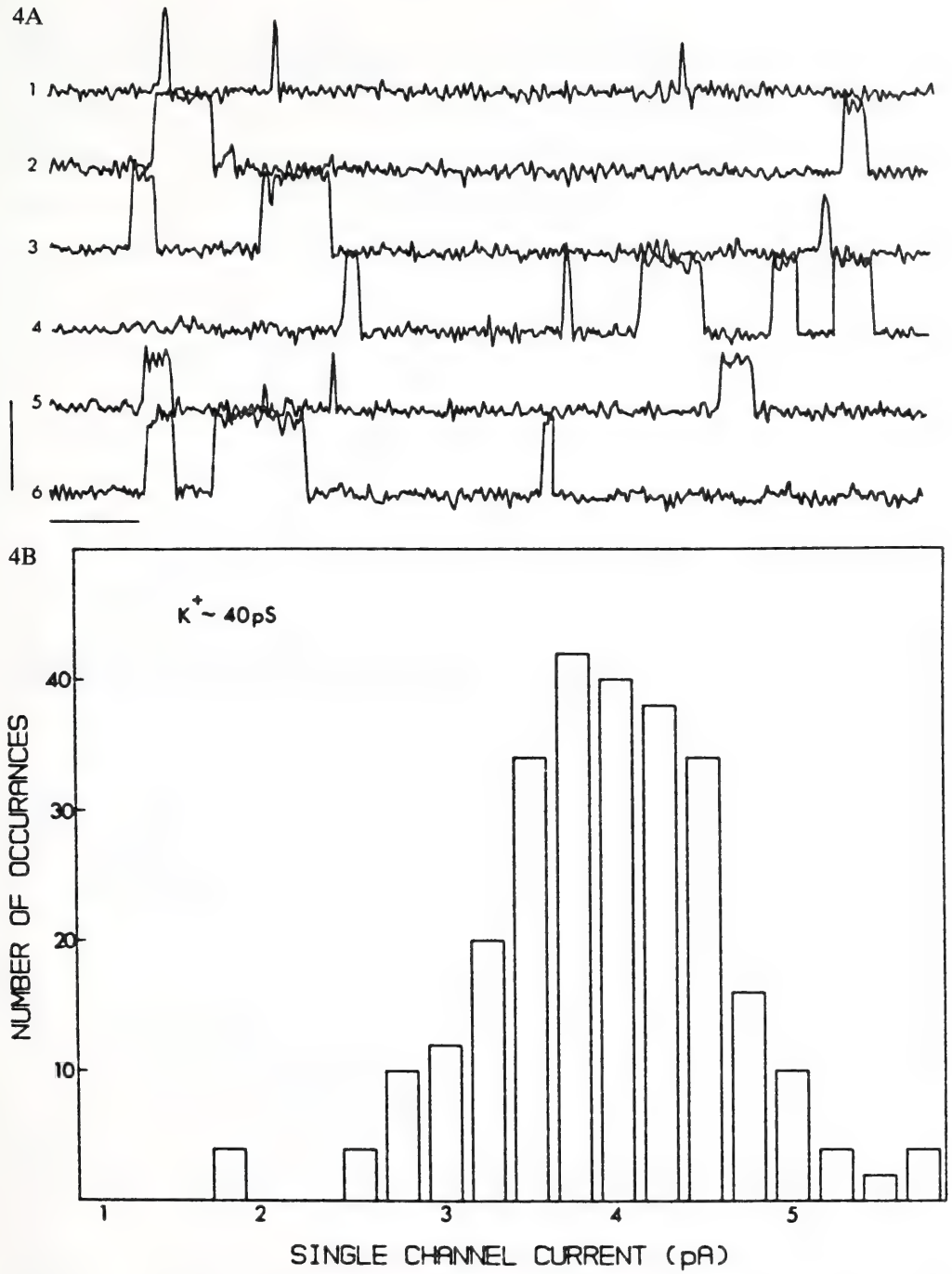


FIGURE 4. Part A shows single channel outward K^+ currents in an inside-out patch held at a potential of +100 mV relative to the grounded bathing solution. The pipette and bath solutions were 100 mM KCl and 2 mM $CaCl_2$ buffered with 5 mM Hepes to pH 5.80 ± 0.05. Current and time calibrations are 4 pA and 10 msec repsectively. The numbers refer to consecutive traces acquired in an event-triggered mode. Part B shows an amplitude histogram for *Asclepias tuberosa* K^+ channels with data from 310 events divided into 0.25 pA bins.

was used as an impermeable anion the reversal potential was 78 ± 6 mV with 100 mM Na^+ gluconate in the bathing solution and NaCl in the pipette, while it was −75 ± 5

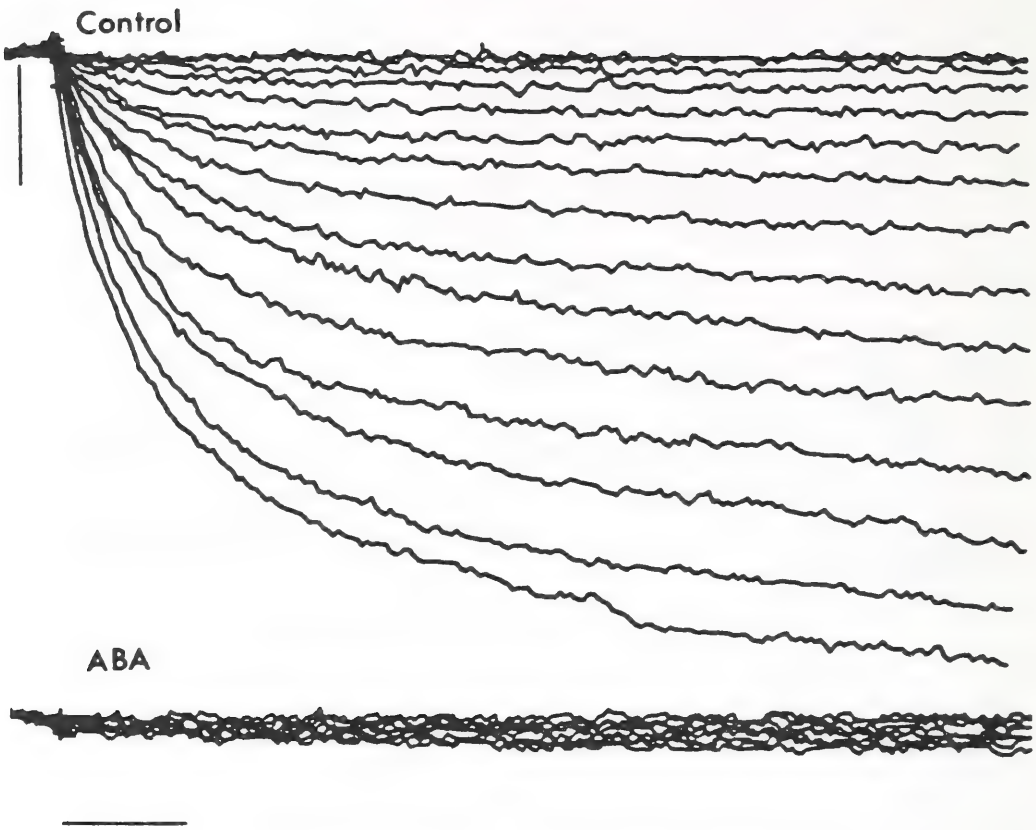
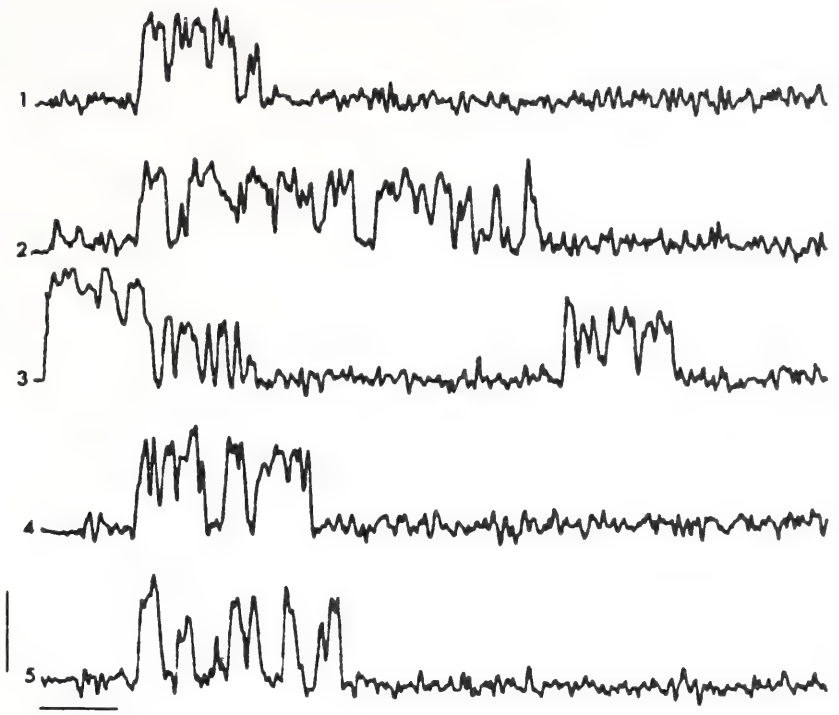


FIGURE 5. Whole-cell inward Cl^- currents in *Asclepias tuberosa* protoplasts for voltage steps of -10 to -150 mV (10 mV increments) before and after addition of 100 uM abscisic acid (ABA). The pipette and bath solutions were 100 mM CsCl and 2 mM $CaCl_2$ buffered with 5 mM Hepes to pH 5.80 ± 0.05. Current and time calibrations are 100 pA and 20 msec.

mV with 100 mM K^+ gluconate in the pipette and a bathing solution of 100 mM KCl. Such data unambiguously identifies these as Cl^- currents, and also defines the Cl^-/cation selectivity ratio as about 20:1. Inward Cl^- currents were inhibited by 10 uM Zn^{++} and 100 uM ethacrynic acid, both known to block anion channels in several animal cells (10). Application of the plant hormones IAA and 2,4-D to the bathing solution at a final concentration of 100 uM) had no effect on these Cl^- currents, whereas 100 uM ABA suppressed them in 6 of 10 cells examined.

Figure 6A illustrates single channel Cl^- currents in *Asclepias tuberosa* for an excised inside-out patch at a potential of -100 mV (traces have been inverted to show inward currents upward for comparison with single-channel K^+ currents). Extrapolation of single-channel currents gave reversal potentials comparable to those for whole-cell data. As for whole-cell Cl^- currents, activity was inhibited by Zn^{++} (Figure 6B). In contrast to single-channel K^+ currents in *Asclepias tuberosa*, single-channel Cl^- currents in the majority of protoplasts were quite nonuniform, suggesting there may be one or more open states. Figure 7 shows the amplitude histogram for Cl^- channels from a patch with relatively uniform openings, with data from 717 events divided into 0.5 pA bins. Single channel conductance in 6 such patches averaged 100 ± 17 pS. Application of 100 uM ABA decreased the mean open time of *Asclepias tuberosa* Cl^- channels, but the hormones IAA and 2,4-D had no effect.

6A



6B

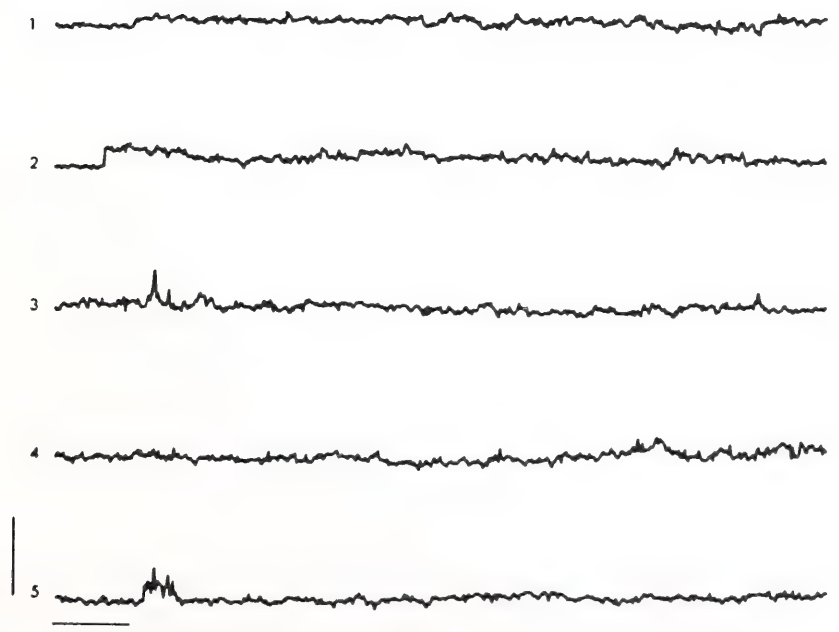


FIGURE 6. Part A shows single channel inward Cl^- currents (shown as upward, reversing the usual convention for visual comparison with the single-channel K^+ currents of Figure 2A) during consecutive traces in an excised inside-out patch held at a potential of -100 mV in symmetrical 100 mM KCl. Part B illustrates similar single-channel data obtained following addition of 10 uM Zn^{++}. Current and time calibrations are 10 pA and 20 msec.

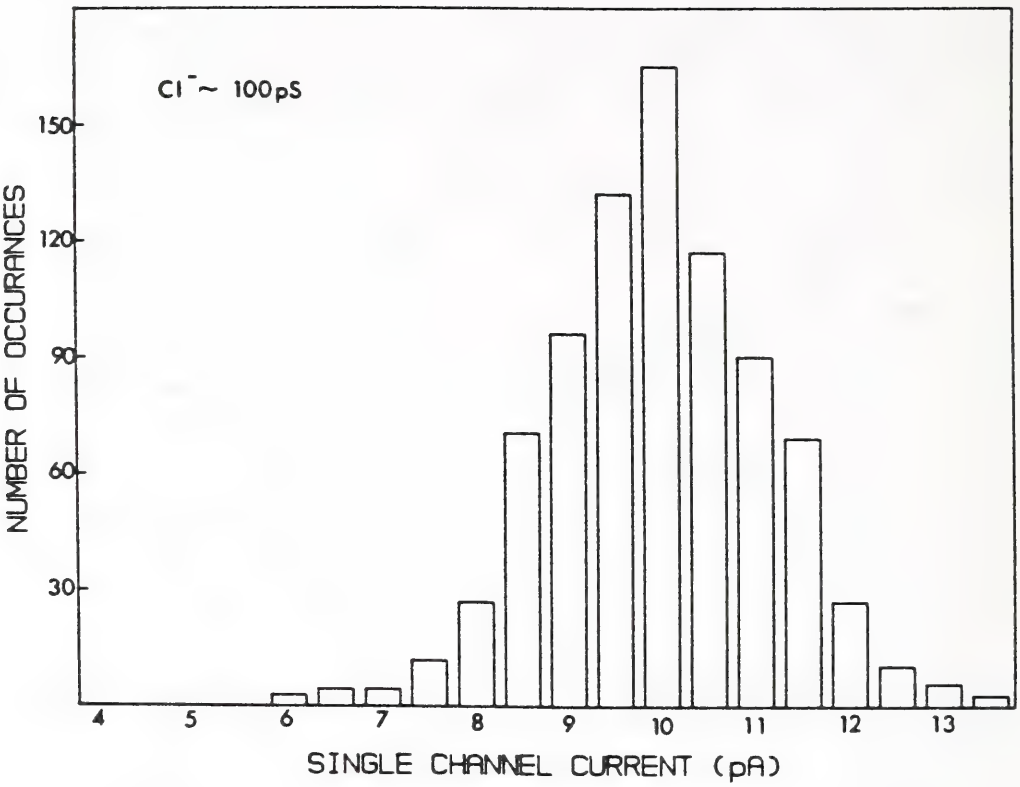


FIGURE 7. The amplitude histogram for Cl^- channels with data from 717 events divided into 0.5 pA bins.

In-Vivo Measurements of Stomatal Aperature

Figure 8 summarizes the results of *in-vivo* stomatal aperature measurements. Exposure of epidermal peels to light in a moist, CO_2-free atmosphere caused the aperature of stomatal pores to increase by $+59.3 \pm 7.3\%$ ($n = 115$) over a period of 2-3 hours. Addition of 10 uM ABA decreased average stomatal aperature by $-37.2 \pm 4.3\%$ ($n = 43$) from the maximal values seen following light exposure, with slightly larger decreases seen in the presence of the well-known K^+ ionophore valinomycin ($-42.8 \pm 4.8\%$; $n = 30$).

In another series of experiments the plant hormone ABA was added in the presence of 100 uM nifedipine, a Ca^{++} channel blocker, or with the external free Ca^{++} reduced to less than 1 uM with an EGTA buffer. Under such conditions no significant change in stomatal aperature could be observed (average changes of $-2.4 \pm 2.2\%$ and $+2.8 \pm 1.5\%$ respectively).

Discussion

Vicia faba guard cell protoplasts have voltage-dependent, ABA-sensitive K^+ channels with a single-channel conductance of 65 ± 5 pS and open time of 1.25 ± 0.30 msec at $+100$ mV. The fact that ABA, but not IAA or 2,4-D, increased the number of single-channel events, prolonged channel open time and induced long-lasting bursts of channel openings suggests that the *Vicia faba* K^+ channel may mediate guard cell responses to environmental factors such as water stress. Increases in channel activity, and thus K^+ permeability, by ABA would facilitate K^+ efflux, leading to osmotically-driven water loss and stomatal closure.

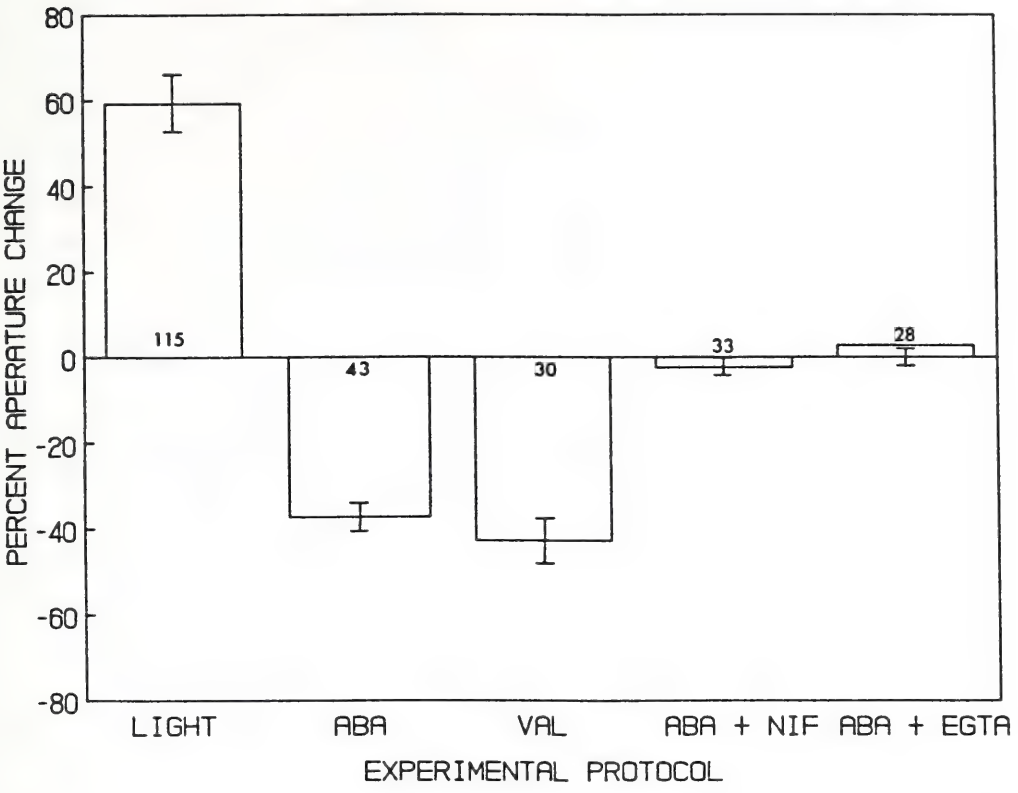


FIGURE 8. Effects of stimuli on stomatal aperature in epidermal peels from *Vicia faba*. The number of observations is given in the lower portion of each group.

One hypothetical model that might explain ABA-dependent regulation of guard cell volume is shown in the upper portion of Figure 9. As for stimulus-secretion coupling in animal cells, increases in intracellular [Ca^{++}] are fundamental. ABA is assumed to interact with a membrane receptor that regulates a Ca^{++} channel (labelled #1). The resulting Ca^{++} influx, perhaps augmented by Ca^{++}-induced Ca^{++} release from internal stores, opens a Ca^{++}-activated K^+ channel either directly, or via an intracellular second messenger. An alternative explanation, shown as #4 in Figure 9, would have ABA interacting with the cell membrane to cause internal Ca^{++} release without the involvement of a Ca^{++}-selective ion channel. However, the fact that the effects of ABA on stomatal aperature were inhibited by removal of external Ca^{++} and by application of nifedipine tends rule out this mechanism.

A possible role for K^+ channels in light-induced swelling in *Vicia faba* guard cell protoplasts is shown in the lower portion of Figure 9. It is assumed that light activates an unknown second messenger system (labelled #1) which, in turn, activates an active H^+/K^+ pump (#2). However, instead of a Ca^{++}-activated K^+ channel being opened, K^+ channels are assumed to close, preventing any short-circuiting of the increase in intracellular [K^+] necessary to create the osmotic gradient necessary for guard cell swelling. In this regard it is interesting that valinomycin and other K^+ ionophores promote stomatal closure and block the normal response to light.

In contrast to *Vicia faba*, a majority of *Asclepias tuberosa* protoplasts contained only Cl^- channels. Single-channel Cl^- conductance was 100 ± 17 pS, though several lower conductance levels were also apparent in the raw data. Cl^- channel activity could be completely blocked by Zn^{++}, ethacrynic acid and by ABA. The role of K^+ and Cl^-

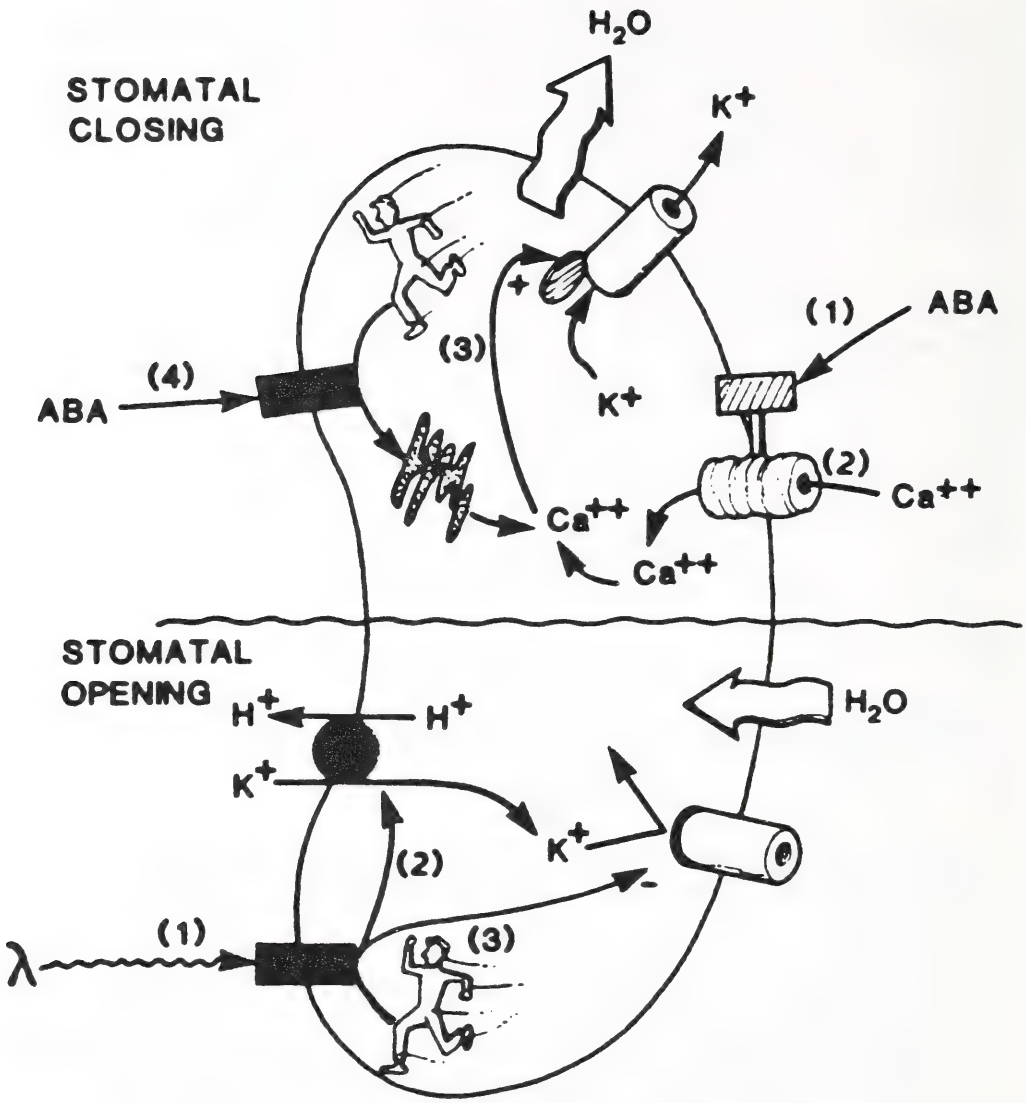


FIGURE 9. A model of ion channel regulation of guard cell volume.

channels in protoplasts derived from long-term suspension-cell cultures of *Asclepias tuberosa* is unknown, however the selective inhibition of a Cl^- channel by ABA raises the possibility that anion-selective channels may also be involved in the responses of plant cells to hormones.

Acknowledgement

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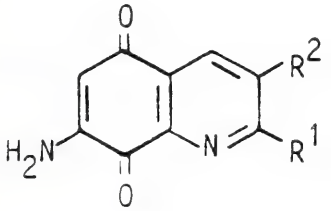
ABSTRACTS

Synthesis of 2-Amino-2-methyl and 2-Amino-3-methylquinoline-5,8-diones (1 and 2). MACKLIN B. ARNOLD, MARK A. HORN AND MOHAMMAD BEHFOROUZ, Department of Chemistry, Ball State University, Muncie, Indiana 47306.——As part of our research toward the total synthesis of antibiotic antitumor agent lavendamycin methyl ester, we prepared aminoquinones 1 and 2. 1 was prepared from 8-hydroxy-2-methylquinoline through a series of transformations. 2 was prepared by the Diels-Alder condensation of 2-acetamido-6-bromo-quinone with 1-(dimethylamino)-1-aza-1,3-diene followed by base hydrolysis.

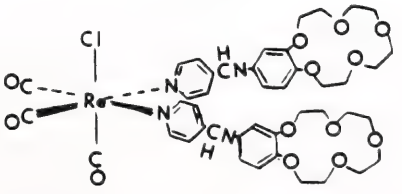


1; $R^1 = CH_3$, $R^2 = H$

2; $R^1 = H$, $R^2 = CH_3$

Rhenium(I) Complexes of Pyridine-functionalized Benzo-15-Crown-5. JOHN D. BAIN, P. ELAINE PARSONS AND BRUCE N. STORHOFF, Department of Chemistry, Ball State University, Muncie, Indiana 47306.——A new ligand with three types of metal binding sites has been synthesized by condensing 4-pyridinecarboxaldehyde with NH_2-derivatized benzo-15-crown-5 (NH_2 group meta and para to the catechol-type oxygens). A Re(I) derivative was obtained by reacting this functionalized crown ether with $Re(CO)_3(CH_3CN)_2Cl$ in a 2:1 ratio. Spectroscopic data (^1H and ^{13}C NMR, IR) indicate that this ligand is bound to the cis positions on rhenium (see I) *via* the less-hindered pyridine nitrogens, thereby providing a bis crown with two additional imine-nitrogen and crown ether sites available to bind other metals.

I



Infrared Studies of Charge-transfer Complexes of Metalloporphyrins and Biologically Active Quinones. JAMES W. BROWN AND J.L. KIRSCH, Butler University, Indianapolis, Indiana 46208.——Charge-transfer complexes are formed between various metalloporphyrins and biologically active quinones, namely Vitamin K and 2,6 Dimethylbenzo-

quinone, a plastoquinone isomer. These quinones have a characteristic carbonyl stretching frequency in the infrared region around 1650-1700 cm^{-1}. Electronic charge is transferred from the large, conjugated porphyrin ring to the quinone. If the quinone acts as an acceptor of electronic charge then this charge is donated to the antibonding orbitals of the carbonyl bond. This donation lowers the bond order of the carbonyl bond and shifts the stretching frequency to a lower wavenumber. Such shifts were observed using FT-IR spectroscopy with the sample in a KBr matrix. The relative shifts were used to determine the relative strengths of the complexes.

The Sometime Peripatetic Carbonyl Function. MARVIN CARMACK, Department of Chemistry, Indiana University, Bloomington, Indiana 47405; MOHAMMAD BEHFOROUZ, Department of Chemistry, Ball State University, Muncie, Indiana 47306; GLENN A. BERCHTOLD, Department of Chemistry, M.I.T., Cambridge, Massachusetts; SAMUEL M. BERKOWITZ, Arizona Chemical, Bay Point, Florida 32407; RALPH P. BARONE, New Jersey; DONALD WIESLER, Department of Chemistry, Indiana University, Bloomington, Indiana 47405.——The action of elemental sulfur (S_8) in hot secondary amine (*e.g.*, morpholine) can cause the stepwise migration of the ketone or aldehyde function along a chain of methylene groups, or around a ring of mostly methylene groups. Thus, under surprisingly mild conditions, 4-heptanone generates a mixture of 4-, 3-, 2-heptanones and heptanal. The active sulfur-nitrogen catalyst is a dithio-*bis*-sulfenamide, and the reactive intermediates from the carbonyl groups are enamines. We discuss a proposal that the central highly reactive intermediates in these complex oxidation-reduction reactions may be thiirenium and/or thiiranium sulfilamines.

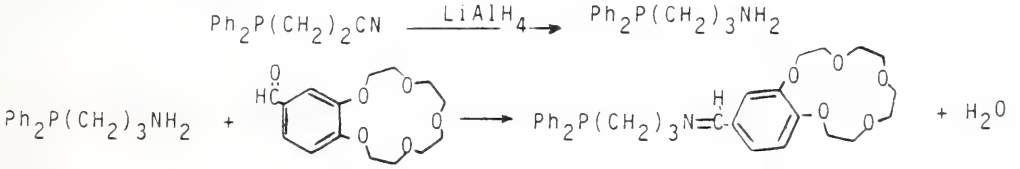
The Reductive Isomerization of αBromoketones. MARVIN CARMACK, Department of Chemistry, Indiana University, Bloomington, Indiana 47405; and JOHN M. CHIGAS, Amoco Oil Research Laboratory, Naperville, Illinois.——Some years ago, R. L. Clarke reported (J. Org. Chem., 1963, 28, 2626) that α-bromoketones, when heated with a thiol (*e.g.*, 1-propane-thiol) and HBr in chloroform, generate a mixture of isomeric enethio ethers, which, upon hydrolysis, yield a mixture of the original ketone without the bromo group and its isomeric ketone with the carbonyl in the position of the original bromo function. We report on studies of this mechanism, which embodies proposed reactive intermediates as S-halothiiranium derivatives. There is a certain parallelism of this acid-catalyzed reaction and the base-catalyzed isomerization discussed in the preceding paper.

Corrosion Potentials of Some Metals and Alloys in the Presence of Sulfur Oxyanions. SHRIKRISHNA W. DHAWALE, Indiana University East, Richmond, Indiana 47374.——Metals and alloys undergo corrosion in sulfur environment. In the present investigation I have studied open circuit potentials of few metals and alloys in the presence of thiosulfate and tetrathionate ions. The open circuit potentials are measured against saturated calomel reference electrode. The potential of corroding surface in an electrolyte measured under open circuit conditions are also known as corrosion potentials. Few studies on the effect of concentration and pH of electrolyte on corrosion potentials are reported here. Under fixed temperature corrosion potential of a metal or an alloy varies with time in a given electrolyte. In some cases it varies in cycles of more anodic and less anodic values with respect to reference electrode. This variation is explained using the passivity model. When passive film is formed there is no contact of metal and its environment. The potential becomes less anodic. Breaking of passive films exposes the metal to the surrounding electrolyte and therefore the potential becomes more anodic making the metal mroe susceptible to dissolution or corrosion. These investigations are helpful in mechanistic studies of corrosion.

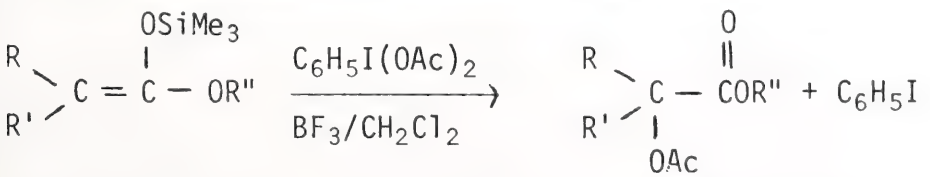
An Easy Way to Draw Enantiomers. HOWARD E. DUNN, University of Southern Indiana, Evansville, Indiana 47712.——An observation of mine is that most people have a great deal of difficulty drawing mirror image isomers of chiral molecules, particularly of the more complex bicyclic systems. I have discovered that most if not all individuals have an unrecognized innate ability to draw mirror image isomers (enantiomers). When one claps his hands or imitates a director of an orchestra his hands and arms move in a synchronous mirror image fashion. By placing a piece of chalk in both hands it is a simple matter to use this natural phenomenon to simultaneously produce mirror image drawings of complex molecules on a chalk board.

Molecular Mechanics (MM2) Calculations and Cone Angles of Phosphine Ligands. GREG L. DURST, MIAN CHIN, PAUL L. BOCK AND JOHN A. MOSBO, Departments of Chemistry, Ball State University, Muncie, Indiana 47306 and James Madison University, Harrisonburg, Virginia 22807.——Molecular mechanics (MM2) calculations were performed on all 54 potentially low energy conformations of 16 phosphines (Ph_3; $PH_{3-n}R_n$ where n = 1 to 3 and R = Me and Et, n = 1 or 2 and R = i-Pr, and n = 1 and R = t-Bu; PMe_2Ph; and $PRPh_2$ where R = Me, Et, i-Pr, t-Bu, and Ph). The results are compared to those previously obtained from MINDO/3 and MNDO calculations, and to experimental data. Single conformer cone angles and weighted average cone angles were calculated from MM2 optimized geometries employing Tolman's general definition, and are compared to Tolman's values, MINDO/3 results and experimental data. The weighted average cone angles are suggested as the best single representation of phosphine ligand sizes.

The Preparation and Study of Phosphine-functinalized Benzo-15-Crown-5. VIDA FARAZI AND BRUCE N. STORHOFF, Department of Chemistry, Ball State University, Muncie, Indiana 47306.——The reaction sequence summarized below has been used to obtain a phosphine derivative of benzo-15-crown-5. The reaction of I with $(PhCN)_2PdCl_2$ provides several complexes as evinced by spectroscopic data. In particular, ^{31}P NMR data are consistent with the formation of both *cis* and *trans* $(I)_2PdCl_2$ in which I is bonded to Pd via the P(III) centers. Spectroscopic evidence also suggests that I can chelate to Pd through the P(III) and imine-nitrogen lone pairs and yield a complex of the stoichiometry $IPdCl_2$.



Hypervalent Idone Oxidation: α-Acetoxylation of Esters Using Iodobenzenediacetate. JEFF M. GARGAS, ATILLA TUNCAY AND ROBERT M. MORIARTY, Department of Chemistry, Indiana University Northwest, Gary, Indiana 46408 and University of Illinois at Chicago, Chicago, Illinois 60607.——Silyl enol ethers react with $C_6H_5I(OAc)_2$ in the presence of a Lewis acid catalyst to yield α-acetoxyesters in good yields with reversal of polarity at α-position. The mechanism of α-functionalization will also be discussed.



R = alkyl or aryl R' = CH_3 or H R" = CH_3 or C_2H_5

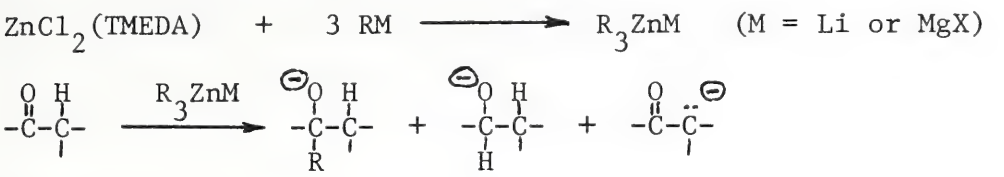
Effect of Temperature on Protein Titration Curves: Theory and Experiment. G.I.H. Hanania and R. F. Sultan, Departments of Chemistry, American University of Beirut, Lebanon, and Indiana University, Bloomington, Indiana 47405.——We have extended application of the discrete-charge model of intramolecular electrostatic interaction in globular proteins to enable computation and interpretation of H^+ titration curves at different temperatures. For this purpose, ferrimyoglobin was titrated with acid and with base, over the range of protein stability, pH 4.0 to 11.0, at four temperatures between 10° and 50°C, and a constant protein concentration and zero salt ionic strength. The experimental results were then compared with theoretical calculations, the latter involving the computation of acid-dissociation constant and effective charge for each of the 60 ionizing groups on the protein; as well as the net protein charge, as a function of pH, ionic strength and temperature. There was good agreement between the calculated and the experimentally determined titration curves, even at 50°C. It may be concluded that any thermally induced perturbations in conformation, or changes in heat capacity of the native protein structure that may occur, will probably have compensating effects, such that the observed net pH and temperature variations are adequately accounted for in thermodynamic terms.

NMR Studies of 2-Hydroxy-2,5-diphenyl-3,4-dichlorofuran in $CDCl_3$. Marie G. Hankins, Warren Hankins, Jeff Klingler and Diana Julian, University of Southern Indiana, Evansville, Indiana 47712.——In 1964, P. S. Anderson reported the NMR spectrum of 2-Hydroxy-2,5-diphenyl-3,4-dichlorofuran in $CDCl_3$. She indicated the appearance of two one-proton singlets for the 5-benzylic and 2-hydroxyl protons, each of which, separated into two peaks on addition of DMSO-D_6. Subsequent studies using solvent effects contradicted Ms. Anderson's original assignment of peaks to the isomers of this compound. Continuing studies on higher resolution instruments indicate that addition of DMSO-D_6 is not necessary to separate the peaks. In fact, an NMR spectrum of the compound run with a 200 MHz Fourier Transform NMR shows two peaks for each of the protons and the C-13 spectrum indicates two peaks for one of the carbons. Two isomers must be present in the original $CDCl_3$ solution.

Evidence for the Enzyme System Responsible for L-Ascorbic Acid Entrapment in Human Erythrocytes. John Henley and Eugene S. Wagner, Center For Medical Education, Ball State Universsity, Muncie, Indiana 47306.——Radioactive labelled ascorbic and dehydroascorbic acids when incubated with human blood migrate irreversibly into red blood cells. Isolation and characterization of the moieties trapped within the cells via IR established both their identities as L-ascorbic acid. Evidence in the form of the degree of *in vitro* entrapment of ascorbic acid as a function of times of incubation and the effect of incubation temperature, an anion recognition site inhibitor, N-ethylmaleimide, on the rate of entrapment will be presented to support the following hypothesis. Ascorbic acid is oxidized on or near the surface of the red blood cell to dehydroascorbic acid which migrates through the lipid portion of the cell wall and is reduced back to ascorbic acid within cell by an enzyme complex such as glutathione reductase. The resulting L-ascorbic acid can not pass through the cell wall and is therefore entrapped.

The Reaction of Triorganozincate Reagants with Carbonyl Compounds: Addition versus Reduction and Enolization. Richard A. Kjonaas and Kimberly K. Clingerman, Department of Chemistry, Indiana State University, Terre Haute, Indiana 47809.——A variety of carbonyl compounds treated with triorganozincate reagents in an attempt to compare these reagents with other carbanion type organometallic reagents in their ability to transfer an R group without giving the usual side reactions—deprotonation and transfer of an

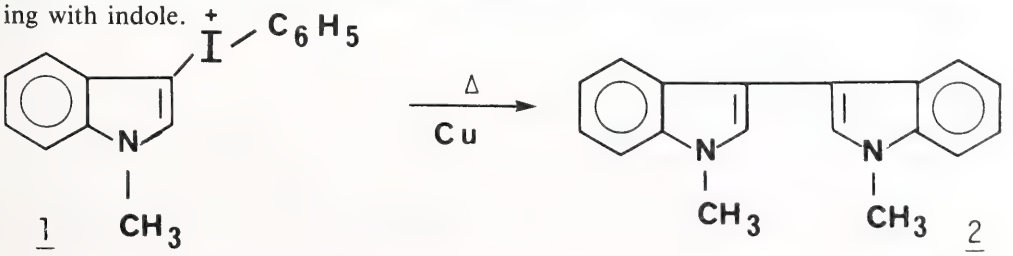
electron followed by a hydrogen atom. The zincate reagents appear to surpass Grignard reagents in their ability to resist these two alternative reaction paths.



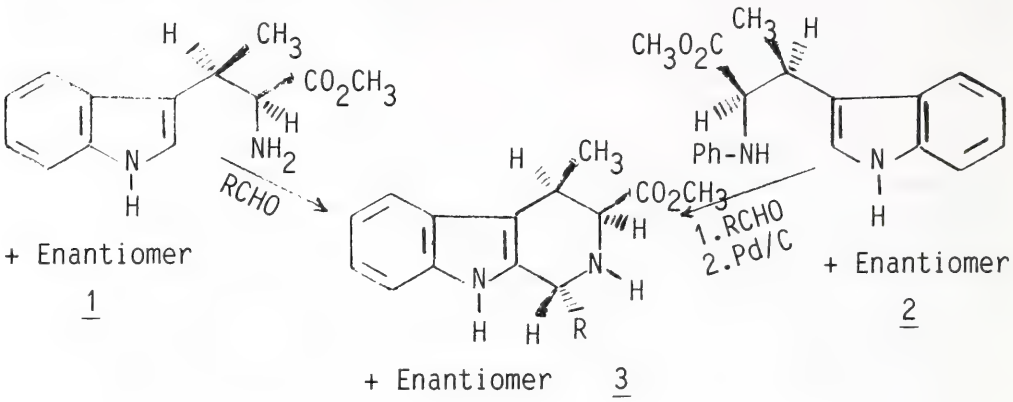
Total Synthesis of a 6-Methyl-A-seco-A,19-dinorsteriod, MIKE MUNCHHOF AND BEN NASSIM, Department of Chemistry, Indiana University Southeast, New Albany, Indiana 47150.——"Dinordrin" a previously synthesized A,19-dinor steroid has shown strong contraceptive properties. In other studies, it has been found that, in general, addition of a methyl group to the 6-position of steroid skeletones increases their antifertility properties. Thus, a total synthesis of a 6-Methyldinordrin analog was undertaken. Optically active (7aS)-Methyl-4-(phenylsulfonylmethyl) perhydroindan-1,5-dione was treated with methyl-3,6-dioxoheptanote-6-ethyleneketal under basic condition to produce a substitution product which after saponification, decarboxylation and cyclization yielded 4-nor-3,5-secoester-9-ene-2,5, 17-Trione 2-ethyleneketal. The 17-ketone was then selectively ketalized and the product was methylated at the 6-position using LDA followed by methyl iodide. The ketals were removed under an acidic condition and the resulting A-seco product is to be annulated to produce the steroid skeletone.

The Effect of Guanidinium Chloride on the Extent of Glutamoyl Peptide Bond Cleavage by *Staphylococcus aureus* Protease V8. MICHAEL D. OBER AND ERIC R. JOHNSON, Department of Chemistry, Ball State University, Muncie, Indiana 47306.——The effect of guanidinium chloride, a common protein denaturant, on the proteolytic activity of *Staphylococcus aureus* Protease V8 (SPV8) was examined. The cleavage extent of glutamoyl peptide bonds by SPV8 was found to decrease with increasing guanidinium chloride concentrations. At 5.0 *M* guanidinium chloride and 37 °C, over 80% of this activity was lost, presumably due to denaturation of the protease. However, a small amount of proteolytic activity persisted at guanidinium chloride concentrations up to 6.0 *M*. SPV8 also appears to be less sensitive to guanidinium chloride denaturation at temperatures below 37 °C. Thus SPV8 appears to retain some proteolytic activity under denaturing conditions, albeit at substantially reduced levels at temperatures above 25 °C. This paper was supported by the Graduate Student Research Fund and the Faculty Research Program of Ball State University.

Synthesis of 3,3′-Biindolyls Using Mixed Iodonium Salts. JULIUS PAWLOWSKI, ATILLA TUNCAY AND ROBERT M. MORIARTY, Departments of Chemistry, Indiana University Northwest, Gary, Indiana 46408 and University of Illinois at Chicago, Chicago, Illinois 60607.——Biindoles have been isolated very recently from marine organisms. Very few synthetic routes to these compounds are available in the literature. We report here the synthesis of N,N′-dimethyl-3,3′-biindolyl 2 using hypervalent iodine methodology starting with indole.



Tetrahydro-β-carbolines Derived from β-Methyltryptophan. David A. Rusk and Mohammad Behforouz, Department of Chemistry, Ball State University, Muncie, Indiana 47306.——1,2,3,4,-Tetrahydro-β-Carbolines of type 3 were prepared by either condensation of the amino acid 1 or its benzyl derivative 2 with aldehydes.



Magnetic Susceptibilities of Acetylacetone and Dipivaloyl-methane Complexes. Briand T. Sanderson and Eugene P. Schwartz, Department of Chemistry, DePauw University, Greencastle, Indiana 46135.——A Johnson Matthey magnetic susceptibility balance constructed on the Evans principle was used to measure the magnetic susceptibilities of solid neutral acetylacetonate complexes of the elements scandium through gallium (excluding titanium) and of the dipivaloylmethanato complexes of vanadium through zinc (excluding titanium). The susceptibilities of certain other second and third row transition elements also were determined. Ring contributions were estimated from the corresponding beryllium and aluminum complexes. The magnetic susceptibility of an ion was found not to depend on the nature of the complex within experimental uncertainty. Mn(III) and Fe(III) complexes were high spin, whereas Ru(III) was low spin. Co(III) was low spin with a high ion magnetic contribution. This ion contribution decreased from Co(III) to Ir(III) and from Co(II) to Zn(II).

Unusual Dielectric Behavior of *bis*-dipivaloylmethanato Complexes of Nickel, Copper and Zinc. Eugene P. Schwartz, Department of Chemistry, DePauw University, Greencastle, Indiana 46135.——Benzene solutions of *bis*-dipivaloylmethanato complexes of copper, nickel, and zinc exhibit non-linear dielectric behavior. Atomic polarizations of the copper and zinc complexes (planar and tetrahedral, respectively) undergo at low concentration a marked decrease with increasing concentration. In contrast, the atomic polarization of the planar nickel complex remains almost constant despite the change in dielectric slope which occurs at high concentrations.

4-Hydroxynonynal in Batten's Disease. A. Sideeq and A.N. Siakotos, Indiana University School of Medicine, Department of Pathology, Indianapolis, Indiana 46223.

A Study of the Adenosine Deaminase Conversion Factor in Pathological Human Blood Serum. Rebecca Smith and Pang F. Ma, Center for Medical Education, Ball State University, Muncie, Indiana 47306.——Previous studies have shown that advanced mammals, including man, exhibit both the large and small molecular forms of the purine catabolic enzyme adenosine deaminase. Through gel filtration column chromotography, an estimation of molecular weights was determined as 200,000 for the large enzyme form and 35,000 for the small enzyme form which can be reversibly converted into the large form when in the presence of a conversion factor. This conversion factor is composed

of a high molecular weight glycoprotein that is aggregated during the conversion process. Previous studies have also shown a tissue specific distribution of the two molecular forms, which would suggest that the conversion factor would be present in higher levels within those tissues where the large form predominates. Knowing that the enzyme follows specific distribution patterns for its two forms, an attempt is made to study the conversion factor levels under various pathological conditions in human blood serum using established gel filtration procedures and simple enzyme assay.

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Mechanisms in Secondary Ion Mass Spectrometry: Sputtering of Preformed Ions and Reactions of Itinerant Protons

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Introduction

Prior to 1981, samples studied by mass spectrometry were limited to those compounds sufficiently volatile to vaporize without thermal decomposition. This precluded the analysis of most biologically important compounds, including steroids, peptides, and proteins. The development of "soft" ionization techniques such as fast atom bombardment (FAB) and secondary ion mass spectrometry (SIMS) have made it possible to analyze such large biomolecules.

The usual procedure of analysis in secondary ion mass spectrometry is dissolution of the sample in a suitable liquid matrix, usually glycerol, followed by bombardment with a 2-7 keV beam of energetic ions. The ions sputtered from the solution into the gas phase are mass-analyzed to produce the SIMS spectrum. One mechanistic model suggests that ionization of neutral molecules takes place in a two-step process, (i) the transfer of sample from the solution into the gas phase, and (ii) ionization of the sample molecules by various reactions, such as protonation to form $(M + H)^+$ or cationization to form, for example, $(M + Na)^+$. SIMS mass spectra usually contain a lower abundance of ions such as $(M + H)^+$ and $(M + Na)^+$ relative to intact cations of ammonium, sulfonium, idonium and other onium salts that exist in the glycerol solution as performed ions. Therefore, it is usually advantageous to convert the sample molecules to an ionic form prior to SIMS analysis. The ionization process then takes place in a single step (the sputtering of a performed ion). This single-step process can be shown to be much more efficient than the two-step process noted above. Selectivity is also enhanced if the derivatization procedure used is specific for a particular functional group in the neutral molecule. This paper describes the development of a derivatization reaction for steroids.

A study of thiamine hydrochloride has been conducted to further understand the protonation process in the selvedge, the region just above the solution/gas-phase interface (2). Even in the SIMS mass spectra of ionic compounds such as the onium salts, there is some evidence for proton transfer reactions in the creation of the fragment ions. The spectrum of thiamine hydrochloride contains ions that result from these reactions.

Methods and Materials

SIMS mass spectra were obtained on a custom-built mass spectrometer, utilizing an Extranuclear quadrupole equipped with a commercial cesium ion gun (Phrasor Scientific). The cesium ions had an energy of 6 keV, with an ion current of 1 uA.

The steroids and Girard's T reagent were obtained from Sigma Chemical Co. and used without further purification. Thiamine hydrochloride was used as received from Mallinckrodt, Inc.

Results and Discussion

Keto-steroids were derivatized with Girard's T reagent, forming a quaternary ammonium salt. The structure and SIMS mass spectrum of derivatized tetrahydrocortisone is given in Figure 1. The mass spectrum contains an abundant ion at m/z 478, corresponding to the intact cation of the derivatized molecule. This ion is well removed in mass

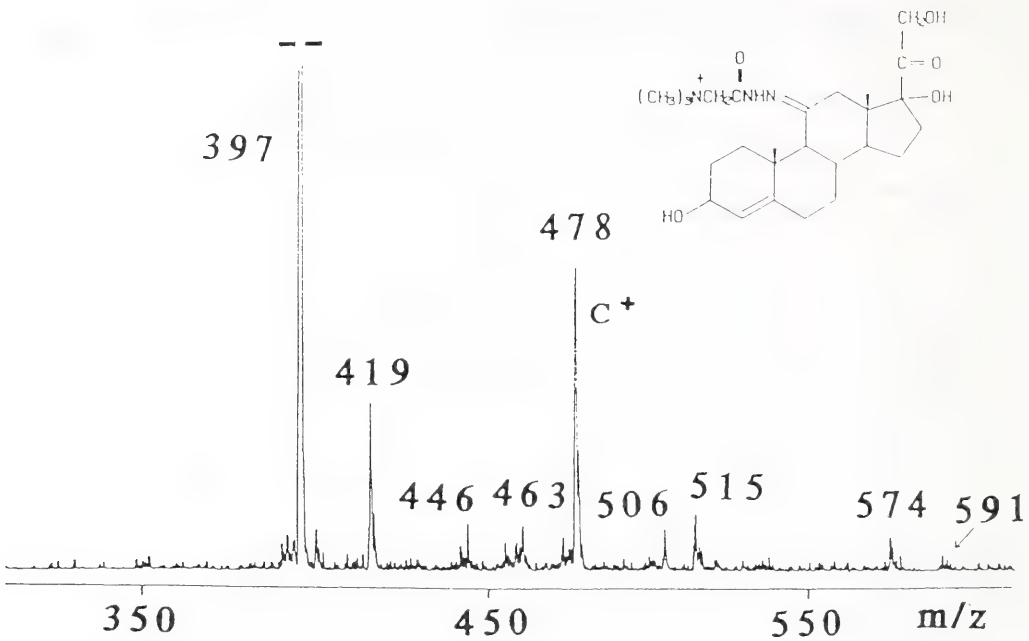

Figure 1. SIMS spectrum of tetrahydrocortisone derivatized with Girard's T reagent.

from the background ions associated with the glycerol matrix. Fragment ions are seen at m/z 463, which arises from the loss of a methyl group from the intact cation, and m/z 446, which corresponds to the subsequent loss of a hydroxy group from the ion at m/z 463. An abundant ion at 419 daltons corresponds to the loss of trimethylamine from the intact caction. This loss of trimethylamine from the intact cation is seen in the mass spectra of all steroids derivatized with Girard's T reagent.

Interestingly, ions of a higher mass than the intact cation are also observed in the spectrum. These correspond to the doubly-derivatized (note the presence of two carbonyl groups in the original structure), but singly-charged, tetrahydrocortisone ion at m/z 591, the fragment ion due to loss of a hydroxyl group from the ion at m/z 574, and an ion from the further loss of trimethylamine at m/z 515. The fact that double-derivatization can occur gives added structural information because one can deduce the number of carbonyl groups on the steroid. The reactions leading to these ions are observed in the mass spectra of all steroids investigated so far. Sensitivity is enhanced by the preformation of the ion, and structural information provided by spectral interpretation.

Thiamine hydrochloride has previously been studied by mass spectrometry using a variety of ionization techniques (1,3,4). Figure 2 shows the processes leading to the most abundant commonly observed fragment ions. The fragmentations observed with all ionization methods are similar with one important difference. Some researchers report a fragment ion at m/z 122, which is the pyrimidine fragment of thiamine, while others report a fragment at m/z 123, the protonated version of the ion at m/z 122. This difference has never been explained. Our study of thiamine hydrochloride was undertaken to provide an insight into the process through which the extra hydrogen is retained in this fragment ion.

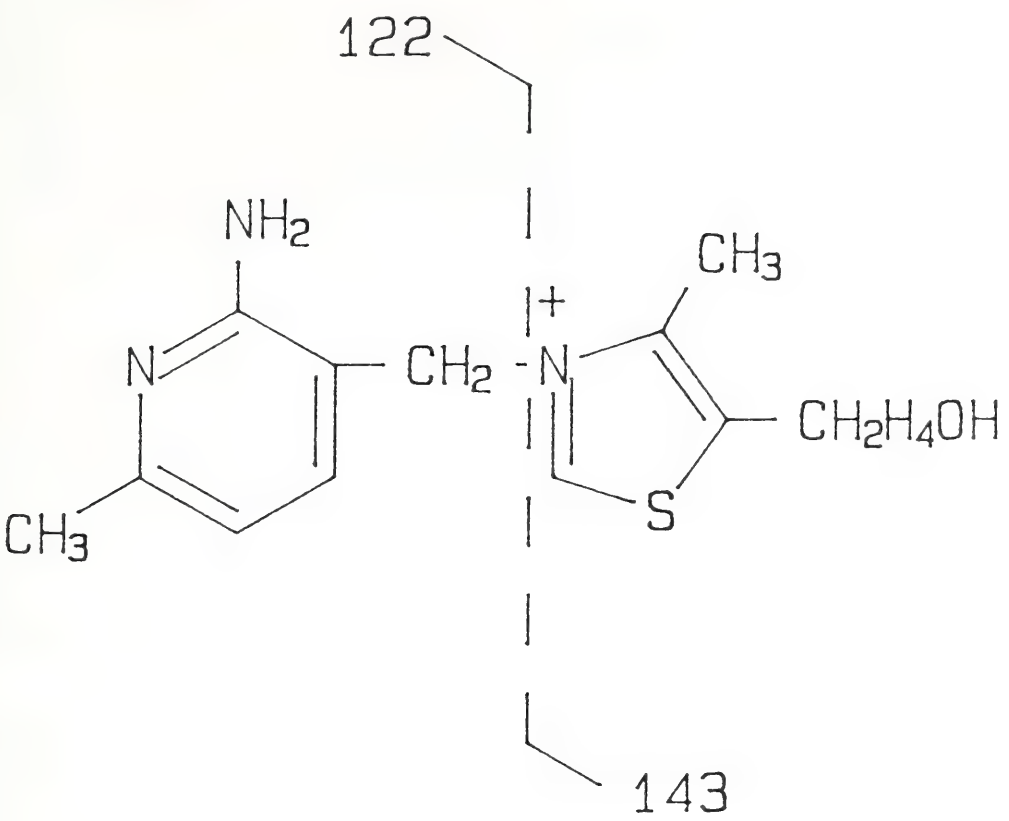

Figure 2. Fragmentation scheme of thiamine hydrochloride.

Figure 3 is the SIMS mass spectra of thiamine hydrochloride using (a) glycerol and (b) sorbitol as matrices. With the sample dissolved in glycerol, the mass spectrum contains the intact cation at m/z 265, the fragment ion at m/z 144 corresponding to the thiazolium ring, and the pyrimidine fragment ion at m/z 122. Also present is an abundant ion at m/z 123, the protonated version of the ion at m/z 122. However, when sorbitol is used as the matrix, the abundance of the ion at m/z 122 is greater than that at m/z 123.

A number of other mass spectra were recorded in which the solvent matrix was rendered more or less acidic with additives such as hydrochloric or camphorsulfonic acid. The ratio of the ions at m/z 122 and 123 were determined in each case. After many such experiments, a correlation between the abundance of these ions and the overall appearance of the spectrum was noted. We suggest that the difference in the spectra is due to a difference in composition of the selvedge region above the solution. With glycerol (Gly) as the matrix, the mass spectrum contains ions at m/z 93 $(Gly + H)^+$ and 185 $(2Gly + H)^+$, as well as those due to thiamine. However, when sorbitol is used as the matrix for dissolution and analysis of thiamine, the mass spectrum does not show any ions corresponding to sorbitol. In all cases in which the mass spectra contained solvent (S) ions $(S + H)^+$, the fragment at m/z 123 is the more abundant. If no solvent ions are seen, the ion at m/z 122 is more abundant. From this information, we can conclude that in the case of thiamine, protonation of the fragment ion occurs in the gas-phase, and the composition of the ion/solvent cluster ions in the selvedge region is the key factor. The ratio of the ions at m/z 123 and 122 is thus an indicator of the relative acidity of the selvedge created by primary ion bombardment.

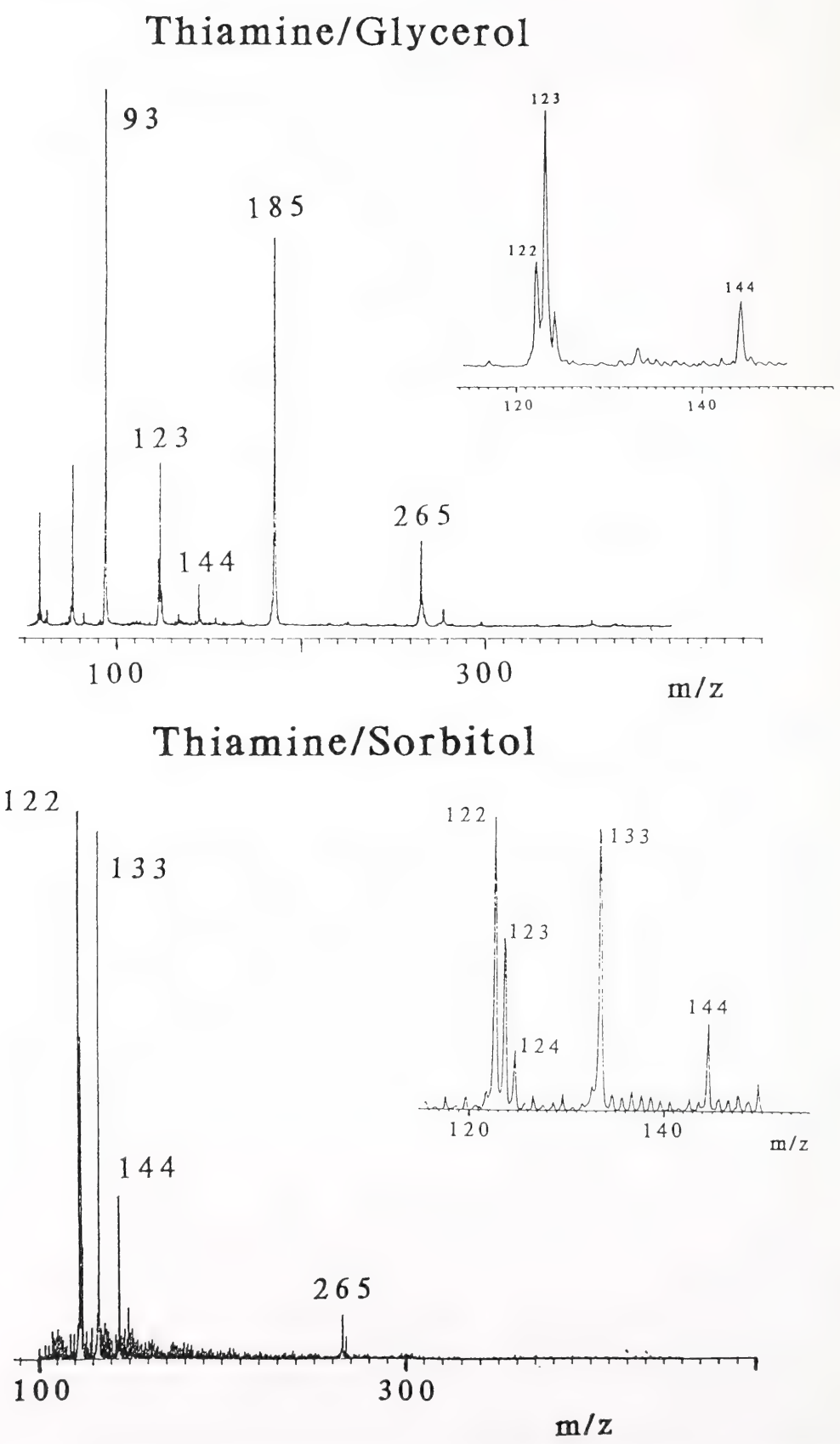

Figure 3. SIMS spectra of thiamine hydrochloride using glycerol and sorbitol as matrices.

Conclusions

It has been shown that the derivatization of keto-steroids with Girard's T reagent to form the quaternary ammonium salt greatly increases the ion yield. Also, the number of carbonyl groups in these steroids can be determined by observing multiply-derivatized species. In a more fundamental study, the protonation of the pyrimidine fragment formed from thiamine hydrochloride has been determined to be a gas-phase process.

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Michael Addition, Isomerization and Derivatives of 2-Carboxamido-3,4-trimethylene-1-indenone[1]

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Indiana University, Bloomington, Indiana 47405

In two recent papers (8,9) we have discussed the synthetic utility of Michael addition of cyanide ion to indenones, and have noted that other Michael reagents add quite well to 2-carboxamido-3-phenylindenone (9). Among these reagents were nitromethane, acetone, malononitrile, bisulfite and hydroxide ion. The addition of cyanide ion to 2-carboxamido-3, 4-trimethyleno-1-indenone (2, Scheme 1), has led to a variety of interesting compounds (5,6,10,11). We wish to report here the results of attempted addition of other Michael reagents to compound 2.

We have shown that β-substituted α-cyanocinnamonitriles can be cyclized in concentrated sulfuric or polyphosphoric acids to indenones, indanones, or mixtures of these, the product depending on the nature of the β-substituents (2,6,7). Cyclization of β-substituted α-cyanocinnamonitriles which do not have a γ-hydrogen atom, such as the β-phenyl or β-*t*-butyl analogs, produce only colored indenones, such as 3-phenyl or 3-*t*-butyl-2-carboxamidoindenones (2,6). Cyclization of ylidenemalononitriles having a γ-hydrogen, as for example 2-cyano-3-isopropylcinnamonitrile, gives a mixture of about one part of colored endocyclic double-bonded indenone, 3-isopropyl-2-carboxamindoindenone, to two parts of white exocyclic double-bonded isomer, 3-isopropylidene-2-carboxamido-1-indanone (6). There is a departure from the above pattern for ylidenemalononitriles with a γ-hydrogen when the β-substituent is part of a fused carbocyclic ring. Cyclization of α-tetrylidenemalononitrile (1a, Scheme 1) yields only the yellow 2-carboxamido-3, 4-trimethyleno-1-indenone (2); whereas 1-benzosuberylidenemalononitrile (1b) gives only the white indanone 3 (7). The formation of only one of two possible double-bond isomers in the cyclization of 1a and 1b is attributed to competing forces: conjugation, which favors the indenone structure, and steric strain, which favors the indanone form.

Discussion

In a continuation of studies of the Michael addition to indenones (9), attempts were made to add acetone to 2-carboxamido-3,4-trimethyleno-1-indenone 2, using various basic catalysts, such as sodium hydroxide, sodium ethoxide or potassium *t*-butoxide. Although colorless solutions were obtained, work-up in the usual way resulted in recovered yellow indenone 2. Addition of sodium hydride to an acetone solution of 2, followed by quenching with acid over ice, produced a light beige compound which could be collected and dried. The infrared spectrum of this compound was similar to that of 2, except for a change in relative intensity and wavelength of the two carbonyl bands, indicating conversion to a non-conjugated ketone, and the disappearance of a conjugated olefinic band. Attempts to recrystallize this compound from ethanol or ether produced a yellow solution on warming, from which 2 recrystallized in high yield. When heated in a capillary tube, the compound went through a phase transition with concomitant color change to yellow-orange between 160-170°, and finally melted at 200-202°, as did a sample of 2 heated concurrently. This compound is therefore the previously unknown unsaturated indanone 4, formed by removal of the vinylogously activated γ-hydrogen of 2 by the acetone anion, to give the resonance-stabilized anion of 4, situated between

three trigonal carbon atoms. Quenching this ion in cold acid produces 4, which reverts to the thermodynamically more stable indenone 2 when heated.

To prove the intermediacy of the carbanion of 4, a trapping experiment was conducted by adding methyl iodide to the colorless solution of 2 and sodium hydride in acetone. The product isolated was the expected 2-methyl-2-carboxamido-4,5-dihydroacenaphthen-1-one, 5a. It exhibited a characteristic ultraviolet spectrum of indanone systems of this type (2,7), as well as the expected pmr characteristics.

In view of the ease with which compound 2 was converted to 5a, further studies on the rearrangement and alkylation of 2 were conducted using various alkylating agents in different solvents. The general procedure consisted of treating 2 with sodium hydride in a solvent. After evolution of hydrogen, an excess of a particular alkyl halide was added to the mixture. The products were isolated by diluting the reaction mixture with water and removing the excess alkyl halide on a steam bath. The product precipitated or was collected by chloroform extraction. Unreacted starting material (2) remained in the basic solution.

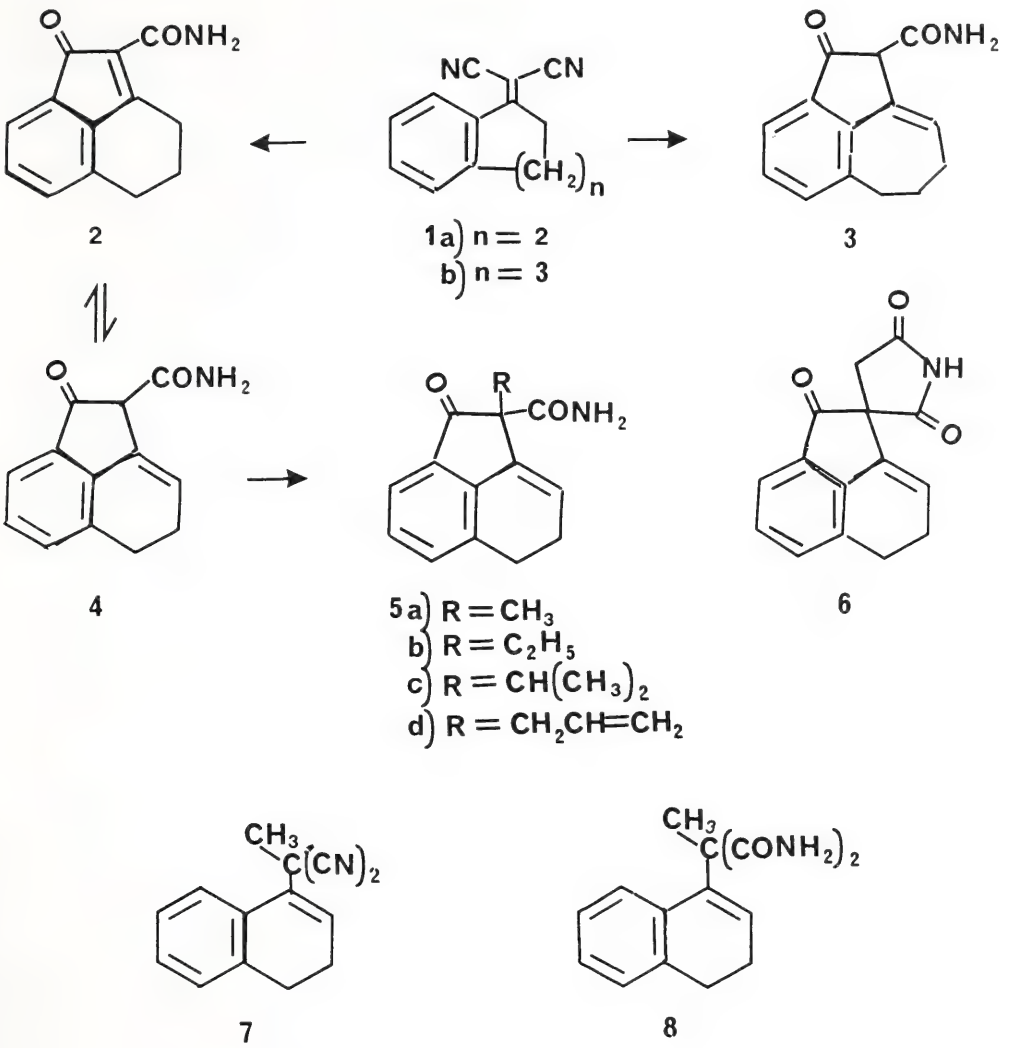
This procedure led to a series of compounds of the general structure 5, except where the alkylating agent was bromoacetate. In this case the product isolated was the spirosuccinimide 6. Compound 6 is formed, following alkylation, by an intramolecular cyclization between the newly introduced carbethoxy group and the carboxamide function. The rearranged alkylated products were characterized by elemental analyses and spectral properties. Certain spectral features are common to all these compounds: 1) the pmr shows a characteristic vinyl triplet (J = 4 cps) between δ 6.4 and 6.0; 2) the infrared spectra exhibit two sharp carbonyl peaks at 5.89 (ketone) and 5.99μ (amide), but 6 shows typical imide absorption at 5.62 and 5.86μ; 3) the ultraviolet spectra have the characteristic unsaturated indanone absorption pattern (2,7) with maxima between 245-248 mμ and shoulders at 255 and 280 mμ.

The alkylation of 2 with primary alkyl halides, such as methyl iodide, ethyl bromide or allyl bromide, appeared to be relatively solvent independent, and the anticipated products 5a, 5b and 5d were obtained in roughly equivalent yields using tetrahydrofuran (THF), dimethylsulfoxide (DMSO) or acetone. However, alkylation with the secondary alkyl halide, isopropyl bromide, seemed to be solvent dependent. Thus 5c was formed in good yield in DMSO, but when the reaction was carried out in THF, 5c was obtained in only 2% yield, and acidification of the mother liquor gave starting compound 2 in 75% recovery. In acetone, only starting material was recovered. The anomalous behavior displayed in the alkylation with isopropyl bromide was undoubtedly due to increased steric hindrance to the approach of the carbanion of 4 to the secondary halide site. The success of this alkylation in DMSO can be attributed to the enhanced reactivity exhibited by carbanions in the solvent (12).

One other distinction was observed with the isopropyl derivative 5c. The pmr spectrum showed the methyl groups of the isopropyl group as two sets of doublets (each coupled with CH) at δ 1.00 and 0.80 (J = 7 cps). The magnetic nonequivalence of the methyl groups arises from the fact that they are attached to a carbon atom adjacent to an asymmetric center. Thus even with free rotation the two methyl groups are in dissimilar chemical environments and have different chemical shifts.

As stated above, compound 5a was synthesized by a two-step process from compound 1a, by first cyclization to 2 and then rearrangement and methylation to 5a. An alternate route to 5a was envisoned by reversing this sequence. Treating 1a with sodium hydride in DMSO or THF gave the desired rearranged methylated product 7 in good yield. The structure of 7 was confirmed by its pmr spectrum, which showed a vinyl triplet at δ 6.33 (J = 4 cps) and a methyl singlet at δ 2.01. Attempted cyclization of 7 with either sulfuric or polyphosphoric acid were unsuccessful. In sulfuric acid only water-soluble products, probably sulfonated derivatives of 7, were formed. In polyphosphoric acid,

Scheme 1

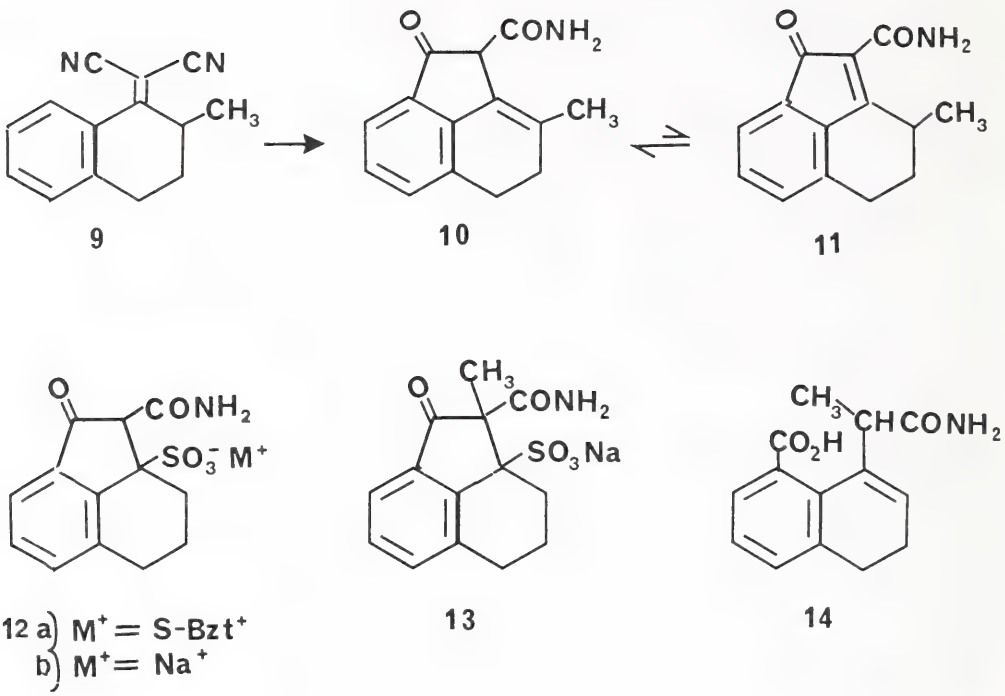


the only product, in low yield, was the diamide 8. The failure of 7 to cyclize under normal cyclizing conditions (7) is analogous to results of Campaigne and Roelofs (4) who were unable to cyclize most saturated malononitrile derivatives. They found that a conjugated double bond system which holds a nitrile group in close proximity to the aromatic ring is required for cyclization.

In order to compare the properties of the 2-methyl derivative 5a with those of its isomer, 2-carboxamido-3-methyl-4,5-dihydroacenaphthen-1-one (10, Scheme 2) we synthesized compound 9, 2-methyl-1-tetrylidenemalononitrile, from 2-methyl-1-tetralone by condensation with malononitrile. As has been noted earlier (3) the usual condensation in refluxing benzene (15) was unsuccessful, but when isopropanol was used as the solvent, this hindered ketone condensed quite well to give 9. There is a distinct difference in the ultraviolet spectra of the two isomers, 7 and 9. Compound 7 shows λ max at 260 mμ ($\epsilon = 6{,}600$) while 9, having the conjugated double-bond system, has λ max at 309 mμ ($\epsilon = 16{,}700$) (2).

Treatment of 9 with concentrated sulfuric acid produced a pale yellow product,

Scheme 2



which did not melt sharply, but had the characteristics of compound 10, as shown by elemental analysis, and the presence of a methyl singlet at δ 1.87 and a methine singlet at δ 4.10 in the pmr spectrum. The isomer, 11, should have a methyl doublet and a split methine proton. The ultraviolet pattern also exhibited the typical indanone pattern quite similar to that of 5a. However, other characteristics indicate that 10 exists in a rapid tautomeric equilibrium at room temperature with a very small percent of 11. The compound is not white, but possesses a slight yellow tinge. Soxhlet extraction of the crude product with diethyl ether, a procedure used to separate yellow indenones from white indanones (2), failed to remove the color. Six recrystallizations from ethanol also failed to decolorize the product, and the melting point range was not narrowed. The compound became deeper yellow as the temperature increased, and melted with decomposition. A thin layer chromatograph of 10 on silica gel gave a yellow band, $R_f = 0.375\text{-}0.50$, eluted with chloroform, but attempted resolution of this band with other solvents was unsuccessful.

Isolation of indanone 10 as the cyclized product from 9 may seem surprising at first glance, since a similar cyclization of 1a (Scheme 1) gives only idenone 2. Although 2 could be converted to the isomeric indanone 4, it was unstable and easily reverted to 2. The change in order of stability between the α, β-unsaturated isomer and the β, γ-unsaturated isomer, produced by the introduction of the γ-methyl group, can be accommodated within the earlier discussion of relative stabilities in the unsaturated 6,6,5-ring system. The greater stability of 2 in comparison to 4 was attributed to the fact that conjugation of the double bond with the ketone and amide carbonyls is of higher energy than steric strain imposed by placing the double bond in the five-membered ring. The introduction of the γ-methyl group brings in a third influence, hyperconjugation of the methyl group with the double bond, which will favor the β, γ-isomer. This is in accord with studies of aliphatic α, β- and β, γ-unsaturated ketones, in which it was found that

introduction of a γ-methyl group into the system shifted the equilibrium toward the β, γ-isomer (13).

Addition of non-carbon nucleophiles to 2 was relatively unsuccessrul. In attempts to add thiocyanate, cyanate, or amines, starting material were recovered in high yield. However, addition of the neutral nucleophile, sodium bisulfite, followed by treatment with S-benzylisothiuronium chloride produced the salt of the corresponding 3-sulfonic acid, 12a (Scheme 2). The corresponding sodium salt (12b) was also obtained by evaporation of a solution of 2 and sodium bisulfite in 50% ethanol. Acidification of a solution of 12b in water led to the isolation of 2, *via* a retro-Michael reaction. In order to prevent the retro-Michael reaction, 12b was converted to the methylated derivative 13 by treating 12b with sodium hybride followed by methyl iodide. However, 13 was also unstable in acid solution, giving 5a.

When indenone 2 was treated with sodium hydroxide in ethanol, followed by methyl iodide, the product obtained after acidification was 2- (8-carboxy-3,4-dihydro-1-naphthyl) propanoamide (14). Indenone 2 had been shown to give 8-carboxy-3,4-dihydro-1-naphthylacetic acid on refluxing aqueous alcholic sodium hydroxide (1) and more recently 3a,4,5,6-tetrahydrosuccinimido [3,4-b) acenaphthen-10-ones substituted at the 10a-position were shown to undergo fascile acid cleavage (8). When a solution of 5a in ethanol was treated with sodium hydroxide at room temperature, it was converted in nearly quantitative yield to 14. This suggests that 5a was an intermediate in the formation of 14 from 2. This may have occurred *via* addition of hydroxide ion to 2, followed by alkylation and elimination of water to form 5a. Indeed, hydroxide has been shown to add to such indenones (9). However, the strong base may have merely deprotonated 2, forming the more stable anion of 4, which was then alkylated and cleaved by excess alkali to give 14.

Experimental

All melting points were determined in open capillary tubes with a Mel-Temp heating block and are corrected. Microanalyses were performed by Midwest Microlab, Inc., Indianapolis, IN. Infrared spectra were determined in potassium bromide disks with a Perkin-Elmer Model 137 Infracord, and were calibrated with polystyrene. Ultraviolet spectra were determined in 95% ethanol with a Bausch and Lomb Spectronic 505 Recording Spectrophotometer. Nuclear magnetic resonance spectra were obtained with a Varian A-60 spectrometer in indicated solvents using tetramethylsilane as an internal standard.

2-Carboxamido-4,5-dihydroacenaphthen-1-one (4).

To a mixture of compound 2 (2) (1.95 g, 9.1 mmoles) in acetone (150 mL) was added sodium hydride (0.55 g of 53.7% NaH in mineral oil, 12.5 mmoles) and the mixture was stirred at room temperature for 30 min. The solution was poured onto 500 mL of cracked ice and the resulting clear solution was acidified with 20% sulfuric acid. The resulting light beige compound was collected by filtration to give 1.80 g (92.5%) of 4. Attempted recrystallization of this compound from 95% ethanol led to the isolation of indenone 2 as shown by color (yellow-orange), melting point and congruent infrared spectrum. When a sample of 4 was heated in a capillary tube, the compound underwent a phase transition between 160-170° with concomitant color change to yellow-orange. At this point this sample could not be differentiated from a sample of 2 heated at the same time; both compounds melted at 201-203°. The infrared spectrum of 4 shows peaks at 2.90, 3.00 and 3.10 (NH), 3.40 (CH), 5.90 (C=O) and 6.00 μ ($CONH_2$). Compound 4 lacks a strong band at 14.4μ which is present in the spectrum of 2. Also in 4, the amide carbonyl is more intense than the ketone carbonyl. The reverse is observed in 2.

2-Carboxamido-2-methyl-4,5-dihydroacenaphthen-1-one (5a).

Sodium hydride (1.5 g of 53.7% NaH in mineral oil, 33.5 mmoles) was slowly added to a mixture of compound 2 (4.0 g, 18.7 mmoles) in acetone (100mL) in an Erlenmeyer flask fitted with a drying tube (Drierite). This addition was followed by rapid evolution of hydrogen. The suspension was stirred at room temperature for 1 hr and then treated with methyl iodide (10 mL). The solution became clear after 30 min and after 18 hr contained a white precipitate. The mixture was then diluted with water (250 mL) and the excess alkyl halide and acetone were removed on a steam bath. The solution was cooled and the precipitate was collected by filtration to give 4.4 g (100%) of crude product, melting at 181-188°. Recrystallization from 95% ethanol, after treatment with activated charcoal, yielded white crystals: mp 200-202°; ir 2,90 and 3.14 (NH), 3.40 (CH), 5.89 (CO), 5.99 ($CONH_2$), and 6.29 μ (phenyl); uv (λ max) 245 (ϵ 21,900), 255-260 (shoulder, ϵ 17,100), and 280 mμ (shoulder, ϵ 6,820); pmr (DMSO-d_6) δ 7.45 (3H, multiplet, aromatic), 7.1 (2H, broad singlet, $CONH_2$), 6.01 (1H, triplet, J = 4 cps, vinyl), 3.00-2.50 (4H, multiplet, methylene), and 1.48 (3H, singlet, methyl).

Anal. Calcd for $C_{14}H_{13}NO_2$: C, 74.01; H, 5.73; N, 6.26. Found: C, 73.94; H, 5.85; N, 6.16.

Compound 5a was also prepared using THF as solvent. After evaporation of the excess alkyl halide and THF, the desired product precipitated on cooling the solution, and was collected by filtration to give 62.4% of material melting at 190-194°, with an infrared spectrum identical with that of 5a above.

Compound 5a was also prepared using η-butyl lithium (1.6 M in hexane, Foote) as the base and dry dioxane as the solvent. Isolation of the product as above gave 77% of material, melting at 191-194°, with an infrared spectrum identical to compound 5a.

2-Carboxamido-2-isopropyl-4,5-dihydroacenaphthen-1-one (5c).

Sodium hydride (0.75 g of 53.7% NaH in mineral oil, 17.2 mmoles) was added to a suspension of compound 2 (2.13 g, 10 mmoles) in DMSO (50 mL). The solution was stirred at room temperature until evolution of hydrogen ceased. After 30 min isopropyl bromide (5 mL) was added and the reaction was maintained at room temperature for 48 hr. The solution was diluted with water (350 mL), the excess alkyl halide removed on a steam bath, the solution cooled, and the product collected by filtration to give 1.95 g (76.5%) of material, melting at 175-180°. Recrystallization from 95% ethanol, after treatment with activated charcoal, gave an analytical sample: mp 185.5-187°; ir 2.90 and 3.14 (NH_2), 3.37 broad (CH), 5.89 (CO), 5.99 ($CONH_2$) and 6.29μ (phenyl); uv (λ max) 248 (ϵ 21,300), 256-260 (shoulder, ϵ 16,800), and 280 mμ (shoulder, ϵ 7.110); pmr ($CDCl_3$) δ 7.5-7.1 (3H, multiplet, aromatic), 6.95 and 6.10 (1H, each, broad singlet, $CONH_2$), 6.37 (1H, triplet, J = 4 cps, vinyl), 3.15-2.30 (5H, multiplet, aliphatic), 1.00 (3H, doublet, J = 7 cps, methyl), 0.80 (3H, doublet, J = 7 cps, methyl).

Anal. Calcd for $C_{16}H_{17}NO_2$: C, 75.29; H, 6.66; N, 5.49. Found: C, 75.01; H, 6.79; N, 5.73.

Attempted preparation of compound 5c in THF afforded 2% of the anticipated product and acidification of the aqueous phase led to the isolation of starting indenone 2 in 76.5% recovery, while the reaction in acetone led only to the isolation of starting indenone 2 (63%), as evidenced by congruent melting points and infrared spectra.

2-Carboxamido-2-allyl-4,5-dihydroacenaphthen-1-one (5d).

Sodium hydride (0.52 g of 50% NaH in mineral oil, 10.8 mmoles) was carefully added to a suspension of compound 2 (2.13 g, 10 mmoles) in THF (30 mL) in a Erlenmeyer flask fitted with a drying tube (Drierite). The mixture was stirred at room temperature for 1 hr, after which allyl bromide (5 mL) was added and the mixture stirred at room temperature for 24 hr. The solution was then diluted with water (200 mL), ex-

cess alkyl halide and THF were removed on a steam bath, and the aqueous phase extracted with three 200 mL portions of chloroform. The chloroform layer was dried (magnesium sulfate), treated with activated charcoal, filtered, and concentrated at reduced pressure. The resulting oil crystallized from ethyl acetate-cyclohexane to give 5d (1.60 g, 63%), melting at 103-104°. Recrystallization from ethyl acetate-cyclohexane gave an analytical sample, mp 105.5-106.5°: ir 2.82 (NH), 3.18 (NH), 5.90 (CO), 5.99 ($CONH_2$), and 6.27 μ (phenyl); uv (λ max) 247 (ϵ 21,500), 256-260 (shoulder, ϵ 17,300), and 280 mμ (shoulder, ϵ 6,330); pmr ($CDCl_3$) δ 7.60-7.18 (3H, multiplet, aromatic), 6.75 (2H, broad singlet, $CONH_2$), 6.30 (1H, triplet, J = 4 cps, ring vinyl), 5.90-4.80 (3H, complex multiplet, vinyl side chain), 3.15-2.5 (6H, multiplet, methylene).

Anal. Calcd for $C_{16}H_{15}NO_2$: C, 75.89; H, 5.93; N, 5.53. Found: C, 75.97; H, 6.11; H, 5.49.

This compound was also prepared in acetone to give 41.5% of material, melting at 102-103.5°, possessing an infrared spectrum identical to the above.

Spiro [4',5'-dihydroacenaphthen-1'-one-2',3-succinimide] (6).

Sodium hydride (1.10 g of 50% NaH in mineral oil, 22.9 mmoles) was added to a suspension of compound 2 (4.26 g, 20 mmoles) in THF (100 mL) in an Erlenmeyer flask fitted with a drying tube (Drierite). The mixture was stirred at room temperature for 1.5 hr; then ethyl bromoacetate (3.67 g, 23.7 mmoles) was added and the mixture stirred an additional 100 hr. The solution was poured onto 500 g of ice and the oily solid was collected by filtration and recrystallized from 50% ethanol to give 1.20 g (23.7%) of product, melting 222-227°. Recrystallization from 50% ethanol gave an analyticsl sample of white plates: mp 229.5-231.5°; ir 3.15 (NH), 5.62 and 5.86 (imide CO), 5.80 (CO), and 6.28 μ (aromatic); uv (λ max) 246 (ϵ 20,000), 255-264 (shoulder, ϵ 15,200), and 280 mμ (shoulder, ϵ 5,700); pmr (DMSO-d_6) *ed* 7.42 (3H, multiplet, aromatic), 6.16)1H, triplet, J = 4 cps, vinyl), and 3.05-230 (6H, multiplet, methylene).

Anal. Calcd for $C_{15}H_{11}NO_3$: C, 71.15; H, 4.35; N, 5.53. Found: C, 70.91; H, 4.59; N, 5.44.

1-(1',1'-Dicyanoethyl)-3,4-dihydronaphthalene (7).

Sodium hydride (1.37 g of 50% NaH in mineral oil, 28.5 mmoles) was slowly added to a solution of 1a (5.3 g, 27.3 mmoles) in DMSO (25 mL) in an Erlenmeyer flask fitted with a drying tube (Drierite). The solution was stirred for 1.5 hr at room temperature. Methyl iodide (4.2 g, 2.95 mmoles) was then added and the solution stirred an additional 14 hr, poured into 200 mL of water and the resulting solid collected by filtration to give 5.2 g (91%) of 7, melting at 50-52°. Compound 7 was also prepared in an analogous fashion using tetrahydrofuran as solvent to give 80% of 7, mp 51-52°. Recrystallization from 50% ethanol afforded white crystals: mp 56-57°; ir 3.42 (CH) and 4.48μ (CN); uv (λ max) 212 (ϵ 13,700), 218 (ϵ 13,900), 225 (ϵ 20,400), and 260 mμ (ϵ 6,600); pmr ($CDCl_3$) δ 7.3 (4H, multiplet, aromatic), 6.33 (1H, triplet, J = 4 cps), 2.5 (4H, multiplet, methylene) and 2.01 (3H, singlet, methyl).

Anal. Calcd for $C_{14}H_{12}N_2$: C, 80.74; H, 5.81; N, 13,46. Found: C, 80.68; H, 5.89; H, 13.33.

1-(1',1'-Dicarboxamidoethyl)-3,4-dihydronaphthalene (8).

Compound 7 (2.08 g, 10 mmoles) was added to polyphosphoric acid (60 g) and stirred on a steam bath for 24 hr. The dark red resolution was poured into 500 mL of water and the resulting solid (1.0 g, 48%) was collected by filtration. The infrared spectrum of this compound showed that it was identical to starting material. The mother liquor was extracted with three 300 mL portions of chloroform. The chloroform was dried (magnesium sulfate), decolorized (Norit), and concentrated at reduced pressure.

The resulting oil was recrystallized from ethyl acetate-cyclohexane to give 0.58 g of white crystals (24%): mp 182-183°; ir 2.9-3.15 (broad, NH_2), 3.42 (CH), 6.00 ($CONH_2$), and 6.05 μ ($CONH_2$).

Anal. Calcd for $C_{14}H_{16}N_2O_2$: C, 68.87; H, 6.55; N, 11.47. Found: C, 68.85; H, 6.75; N, 11.38.

2-Methyl-1-tetrylidenemalononitrile (9).

2-Methyl-1-tetralone was prepared in 57% yield by the method previously reported (14), bp 95-100° (1.0 mm) [lit (14) bp 115-116° (2.5 mm)]: ir 3.41 (CH), 5.95 (CO) and 6.25μ (phenyl); pmr ($CDCl_3$) δ 7.76 (1H, multiplet, aromatic), 7.05 (3H, multiplet, aromatic), 2.81-1.32 (5H, multiplet, methylene) and 1.00 (3H, doublet, J = 7 cps, methyl). The condensation with malononitrile was accomplished following a modification of the method of Mowry (15), but replacing benzene with isopropanol as solvent. 2-Methyl-1-tetralone (22.5 g, 14.1 mmoles), malononitrile (20 g, 30 mmoles), ammonium acetate (2g), and acetic acid (5 mL) were refluxed in isopropanol (190 mL) for 24 hr. The solution was concentrated to dryness at reduced pressure and the solid was dissolved in chloroform, washed with water, dried (magnesium sulfate), treated with activated charcoal, and concentrated at reduced pressure on a rotary evaporator. The resulting oil was crystallized from 95% ethanol to give 12.3 g (42%) of 9, melting at 77-79°. Recrystallization from 95% ethanol gave an analytical sample: mp 84.5-85.5°; ir 3.41 (CH) and 4.52μ (CN); uv (λ max) 235 (ϵ 7,600) and 309 mμ (ϵ 16,700).

Anal. Calcd for $C_{14}H_{12}N_2$: C, 80.74; H, 5.81; N, 13.46. Found: C, 80.44; H, 5.90; N, 13.54.

2-Carboxamido-3-methyl-4,5-dihydroacenaphthen-1-one (10).

A solution of 9 (9.9 g, 50 mmoles) in 100 mL of conc. sulfuric acid was warmed on a steam bath to 60°, removed, and the temperature rose to 76° in 10 min. When the temperatures began to drop, the solution was heated to 80°, allowed to cool to 40° (1 hr) and then poured onto 1 Kg of cracked ice. After 24 hr the resulting solid (yellow-white) was collected by filtration to give 9.52 g (84.2%) of crude product, mp 196-201°. The product was subjected to Soxhlet extraction with diethyl ether. The yellow color was not removed from the material in the cup, and evaporation of the ether yielded a material identical to that in the cup as shown by infrared comparison. Six recrystallizations from 95% ethanol (Norit) afforded a yellowish-white material which showed a narrow band on tlc (silica gel) eluted with chloroform, R_f .375-.50. The compound partially melted at 200° and completely melted at 217-220° with decomposition. This material was assigned structure 10 based on the following spectroscopic properties, but the slight yellow color is attributed to a small amount of tautomer 11, which exists in equilibrium with 10: ir 2.92 and 3.15 (NH_2), 3.42 (CH), 5.88 (CO), 6.00 ($CONH_2$) and 6.28 μ (phenyl); uv (λ max) 246 (ϵ 15,100), 255-260 (shoulder, ϵ 11,400), 272-275 (shoulder, ϵ 8,800), and 284-287 mμ (shoulder, ϵ 5,100); pmr (DMSO-d_6) δ 7.80 (1H, broad singlet, amide), 7.33 (4H, multiplet, aromatic and amide), 4.10 (1H, singlet, methine), 3.10-2.25 (multiplet, methylene), and 1.87 (3H, singlet, methyl).

Anal. Calcd for $C_{14}H_{13}NO_2$: C, 74.01; H, 5.73; N, 6.17. Found: C, 74.10; H, 5.66; N, 6.13.

S-Benzylisothiuronium 2-Carboxamido-3,4-trimethyleno-1-indanone-3-sulfonate (12a).

A mixture of 2 (2.0 g, 9.4 mmoles), sodium bisulfite (2.0 g, 19.2 mmoles) in 100 mL of 50% aqueous ethanol was warmed on a steam bath until a clear amber solution was formed (5 min). The solution was concentrated to a volume of 50 mL on a hot plate under a stream of air, cooled in an ice bath, and the clear amber solution treated with

S-benzylisothiuronium chloride (9.4 mmoles) to give 2.85 g (66%) of a beige precipitate, mp 141-144°. Recrystallization from 50% ethanol afforded colorless crystals: mp 146-147°; ir 2.80 and 3.0-3.4 broad (H-bonding), 5.85 (CO), 5.99 ($CONH_2$), and 8.1-8.8 μ (SO_3).

Anal. Calcd for $C_{21}H_{29}N_3O_5S_2$: C, 54.90; H, 5.00; N, 9.15; S, 13.96. Found: C, 54.59; H, 4.99; N, 9.08; S, 13.77.

The sodium salt of the sulfonic acid could also be isolated; however, an analytical sample could not be prepared. Thus indenone 2 (33 g, 15.5 mmoles) and sodium bisulfite (19 g, 18.2 mmoles) were placed in 200 mL of 50% ethanol. The mixture was heated on a hot plate and concentrated to 50 mL. A chloroform extract of the clear amber solution was dried (magnesium sulfate), decolorized with Norit, and concentrated to dryness at reduced pressure to give 0.5 g of starting material. The aqueous phase was decolorized with Norit and concentrated to dryness at reduced pressure to give 47.5 g of sodium 2-carboxamido-3,4-trimethyleno-1-indanone 3-sulfonate (12b), mp 185-187° (dec). Acidification of a colorless solution of the sodium sulfonate salt 12b in water afforded a yellow solution from which indenone 2 was isolated. Recrystallization of 12b from 95% ethanol-THF (a small amount of undissolved material was removed by filtration) afforded white crystals: mp 187-188° with decomposition to an orange material which is undoubtedly indenone 2 formed by a retro-Michael reaction on heating; ir 2.8-3.4 (H-bonding), 5.80 (CO), 6.00 ($CONH_2$), and 8.2-8.6μ (broad SO_3).

A good elemental analysis could not be obtained for this compound.

Anal. Calcd for $C_{13}H_{12}NO_5SNa \cdot H_2O$: C, 46.50; H, 4.17; N, 4.17. Found: C, 45.87; H, 4.35; N, 4.00.

Sodium 2-Carboxamido-2-methyl-3,4-trimethyleno-1-indanone-3-sulfonate (13).

A solution of 12b (6.34 g, 20 mmoles) in dimethylformamide (50 mL) was treated with sodium hydride (1.20 g of 53% NaH in mineral oil, 27 mmoles) After 20 min methyl iodide (10 mL) was added and the reaction was kept at room termperature for 100 hr. The resuting solid was collected by filtration and washed with ether and chloroform to give 2.75 g (42%) of material which did not melt at 360°. The compound gave a positive test for sulfate with barium chloride, after digestion with nitric acid. The infrared spectrum exhibited peaks at 2.90 (NH), 5.90 (CO), 6.00 ($CONH_2$), and 8.2-8.65 μ (SO_3). The compound was soluable in water and upon acidification afforded 2.5 g (55%) of 5a, as evidenced by congruent infrared spectrum and melting point. The ether-chloroform solution was extracted with water, and acidification of the aqueous phase yielded an additional 0.5 g (11%) of 5a.

2-(8-Carboxy-3,4-dihydro-1-naphthyl)propanoamide (14).

A mixture of indenone 2 (1.4 g, 6.5 mmoles), 10% sodium hydroxide (10 mL) and 95% ethanol (15 mL) was stirred at room temperature until a homogenous amber-colored solution was obtained (30 min). Methyl iodide (5 mL) was added and the solution was stirred an additional 12 hr at room temperature. The reaction mixture was diluted with 200 mL of water, acidified to pH 3 with 20% sulfuric acid, and the excess methyl iodide was removed on a steam bath. The resulting solid was collected, yielding 1.18 g (74%) of 14, mp 190-192°. An analytical sample was prepared by recrystallization from 95% ethanol: mp 197.5-199°; ir 2.90 and 3.05 (NH_2), 3.30-4.00 (H-bonded COOH), 5.88 (COOH, 6.10 ($CONH_2$), and 6.30 μ (phenyl); uv (λ max) 263 (ϵ 7,500) and 305 mμ (ϵ 1,180); pmr (DMSO-d_6) δ 7.29 (3H, multiplet, aromatic), 6.68 (2H, multiplet, amide), 6.29 (1H, triplet, J = 4.5 cps, vinyl), 3.4 1H, quartet, J = 7 cps, methine) 2.8-1.9 (broad multiplet, obscured DMSO, methylene) and 1.21 (3H, doublet, J = 7 cps, methyl). This compound was soluble in sodium bicarbonate.

Anal. Calcd for $C_{14}H_{15}NO_3$: C, 68.55; H, 6.12; N, 5.71. Found: C, 68.75; H, 6.37; N, 5.81.

Compound 14 was also synthesized from 5a by the following procedure. A mixture of 5a (1.4 g, 6.2 mmoles), 10% sodium hydroxide (15 mL) and 95% ethanol (15 mL) was stirred at room temperature for 12 hr, then diluted with 200 mL of water and acidified to pH 3 with 20% sulfuric acid. The solid was collected by filtration to give 1.20 g (74%) of 14, as shown by identical infrared spectrum and melting point.

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Note

[1] Taken in part from a thesis submitted to Indiana University by D.A.T. in partial fulfillment of the requirements for the degree of Doctor of Philosophy, September, 1968. This work was supported in part by a grant from Bristol Laboratories, Syracuse, NY.

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Alkylation and Isomerization of 2-Carboxamido-3-ethyl-1-indenone and 2-Carboxamido-3-ethylidene-1-indanone[1]

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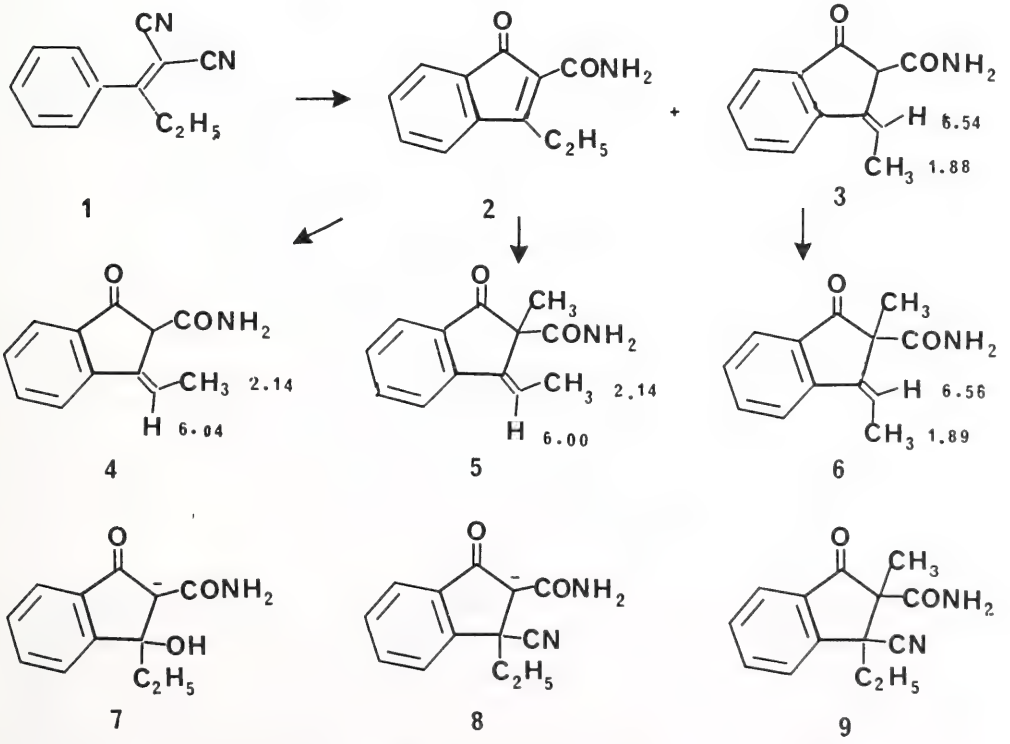
Introduction

We have recently reported on the Michael addition to indenones (4) and on the isomerization and alkylation of 2-carboxamido-3,4-trimethyleno-1-indenone (5). This compound is the only example in which the indenone isomer is the sole product from the acid cyclization of an ylidenemalononitrile having a gamma-hydrogen (13). The isomeric indanone has been obtained as an unstable white product which reverts to the parent indenone on melting or attempted recrystallization (5). Generally, the cyclization of an ylidenemalononitrile having a gamma-hydrogen, such as 2-cyano-3-ethylcinnamonitrile (1, Scheme 1) yields a mixture of the yellow indenone (2) and white indanone (3) in a ratio of about one part of 2 to two or three parts of 3 (1). These isomers can be separated by different solubilities in ether. The yellow indenone 2 melted at 150°, turned white and solidified, and melted again near the melting point of indanone 3, indicating the indanone isomer is the more stable of this pair.

Scheme 1



Discussion

Our previous study (5) would indicate that treatment of either 2 or 3 with sodium hydride and methyl iodide would give the same methylated indanone. However, treat-

ment of 2 in this way gave a product which melted 40° higher than the product for similar treatment of 3. The elemental analyses of these two products were the same, consistent with a methylated derivative, and the ultraviolet spectra were similar and typical of comparable unsaturated indanones (1,5). The infrared spectra were similar, but showed distinct differences in the finger-print region.

The difference in structure of these two isomeric indanones was established by pmr spectroscopy. Although both compounds exhibited aromatic and amide multiplets centered at δ 7.5 and a singlet for the C_2 methyl group at δ 1.43, the difference was in the chemical shifts of the vinyl hydrogen and methyl groups. The product derived from 2 had a methyl doublet (J = 7.5 cps) at δ 2.14 and a vinyl quartet (J = 7.5 cps) at δ 6.00. The product from 3 had a methyl doublet (J = 7.5 cps) at δ 1.89 and a vinyl quartet (J = 7.5 cps) at δ 6.56. The difference between the two isomers is in the configuration around the ethylidene double bond. The isomer derived from 2 has the methyl group deshielded, compared to the methyl group in the isomer derived from 3, and the reverse is true for the vinyl hydrogens. The configuration of the two isomers was assigned assuming that the substituent that is *cis*-oriented to the carboxamide group at C_2 would be deshielded more than the corresponding *trans*-isomer (7). Consequently the product from 2 is assigned as Z-2-carboxamido-2-methyl-3-ethylidene-1-indanone (5), and that derived from 3 is E-2-carboxamido-2-methyl-3-ethylidene-1-indanone (6).

Now that the chemical shifts of the vinyl hydrogen and methyl protons are known for both geometric isomers of the ethylidene group, the configuration of indanone 3, obtained by cyclization of 1, can be assigned. The chemical shift of the vinyl hydrogen is at δ 6.56 and the methyl group at δ 1.88. These values are almost identical to those of 6. Therefore the configuration of 3 is that shown on Scheme 1, E-2-carboxamido-3-ethylidene-1-indanone. The formation of only one indanone isomer from acid cyclization is surprising, but may depend on steric repulsion between the C_2 substituents and the ethyl group in the transition state. However, a more reasonable explanation involves coordination of one of the acidic methylene protons of the ethyl group with the negative carbonyl group of the amide in the protonated intermediate. Thus elimination of a methylene proton with electron migration and hydrolysis of the resulting vinylamine leads to 3. Since indanone 3 and the methylated indanone 6 have the same configuration, the formation of 6 from 3 must proceed by simple abstraction of the acid C_2 proton by sodium hydride followed by alkylation.

The reason for the formation of the more hindered Z-isomer 5, when indenone 2 is isomerized and alkylated, is not obvious. The base-catalyzed allylic rearrangements of 1-alkenes to either or both *cis* and *trans*-2-alkenes have been discussed by Cram (6), and the same logic can be applied to the base-catalyzed isomerization of 2. It has been found that in kinetically controlled processes the less stable *cis*-isomer is the preferred product, while the more stable isomer is formed in thermodynamically controlled processes. Since proton abstraction from 2 by sodium hydride is an irreversible process, the expected product would be the kinetically controlled product. Proton abstraction would occur most easily from that rotomer of 2 having the gamma-protons most exposed, which is this case leads to the Z-isomer 5.

Since the non-nucleophilic base sodium hydride produced the less stable Z-isomer on rearrangement and alkylation, it was interesting to examine the rearrangement of 2 with the nucleophilic base sodium hydroxide, previously reported (1). Isomerization of 2 in the presence of one equivalent of sodium hydroxide in ethanol, followed by dilution and acidification gave an isomeric white indanone not identical to 3. The pmr spectrum of this substance, obtained in 45% yield, showed a vinyl quartet at δ 6.04 and a methyl doublet at δ 2.14. These chemical shifts are almost identical to the values for the methylated Z-indanone 5, and we have therefore assigned the structure as Z-2-carboximade-3-ethylidene-1-indanone (4, Scheme 1).

Compound 4 exhibited unusual melting point behavior. The white compound melted at 130°, turned yellow and resolidified, melted again at 145°, turned white and resolidified, and melted again at 190°. This behavior is similar to that of 2. Apparently the less stable Z-isomer 4, on heating, isomerizes to the yellow indenone 2, which then isomerizes when melted to form the more stable E-isomer 3.

The mother liquor from the sodium hydroxide isomerization yielded about 20% of 3 contaminated with a trace of yellow 2. Purification gave 3 as shown by congruent infrared spectra and mixture melting point. However, treatment of 3 with sodium hydroxide, as above, for 100 hr led to about 80% recovery of 3, indicating that the E-isomer does not isomerize, or isomerizes only slightly, under basic conditions.

The formation of both the E- and Z-isomers, 3 and 4, from the sodium hydroxide catalyzed isomerization of 2 suggests that a different mechanism than that proposed for sodium hydride isomerization may be involved. Sodium hydroxide is both a base and a nucleophile, so that in addition to proton abstraction, hydroxide may add to the conjugated double bond of 2, followed by elimination of water, which could produce both 3 and 4. Addition of hydroxide to indenones has been shown to occur (4).

Table 1
Principal Ultraviolet Absorption Peaks, in mμ.

A	B	C	D	E
Compound 2				
		229	223	
248	236	236		234
254	258	258	264	254
		274	274	
331	319	320	319	327
Compound 3				
242	225	225	222	225
264	264	264	264	264
273	273	273	273	275
332	318	318	318	318

A. In 95% ethanol. B. In 95% ethanol containing 100 molar excess sodium hydroxide, after 1 hour. C. Same as B after 24 hours. D. Same as B after 120 hours. E. In 95% ethanol containing 10 equivalents of sodium cyanide, after 24 hours.

If sodium hydroxide is abstracting a proton from 2, causing rearrangement to the reasonanced stabilized carbanions of 3 and/or 4, then the ultraviolet spectra of 2 and 3 in ethanolic sodium hydroxide should be identical. However, if hydroxide ion added to the conjugated double bond, a discreet intermediate 7 would be formed, and should be detectable by ultraviolet spectroscopy. The result of ultraviolet spectroscopy studies of 2 and 3 in ethanol, in ethanolic sodium hydroxide over time, and in ethanolic sodium cyanide solution, are summarized in Table 1. The set of data for 3 is relatively simple. The spectrum of 3 in neutral solution (Column A) is slightly changed on the addition of base, causing a hypsochromic shift of the principal peak at 242 to the 225-222 region, and a slight hypsochromic shift of the peak at 332 to 318-320 (Columns B and C). The mid peaks at 264 and 273 are unchanged, even after 120 hours in basic solution (Column D). When 3 is treated with sodium cyanide in alcohol (Column E), the U.V. spectrum

is nearly identical to that in alcoholic hydroxide solution. This indicates that cyanide is a sufficiently strong base to abstract the proton at C_2 to form the anion of 3. The spectrum of this solution after acidification was the same as 3.

The changes in the ultraviolet spectra of 2 in base with time, shown at the top of Table 1, were more complex. The initial change from the neutral indenone (Column A) to that after one hour in basic solution (Column B) is a hypsochromic shift of the 248 and 331 peaks to 236 and 319, and a slight bathochromic shift of the 254 peaks to 258. As time increases from one to 120 hours, the spectrum of 2 changes with two new peaks appearing at 264 and 274 mμ. After 120 hours in hydroxide solution, spectra of 2 and 3 are identical, that of the anion of 3. Column E (Table 1) shows that the U.V. spectrum of 2 with cyanide is quite similar to that of 2 after one hour in base (Column B). Cyanide is known to add readily to similar indenones (2,5), and the U.V. spectrum of anion 8 (Scheme 1) should be very similar to that of 7.

Final and conclusive evidence for the formation of the hydroxide adduct 7 was obtained from the pmr spectra of 2 and 3 in sodium deuteroxide-deuterium oxide solution. The pmr spectrum of the anion of 3 would be expected to exhibit a vinyl quartet and a methyl doublet, whereas the hydroxide adduct 7 would be expected to show a methyl triplet and a methylene quartet. The desired information could be obtained using the aromatic multiplets of 2 and 3, not affected by the base, as internal standards. The spectrum of 3 in sodium deuteroxide-deuterium oxide shows a vinyl proton, which was 1.28 ppm upfield from the aromatic multiplet, and a methyl doublet 5.15 ppm upfield. The integral trace gives a correct ratio (6:1:3) of the combined aromatic-amide protons, vinyl and methyl protons. The spectrum of 2 in sodium deuteroxide-deuterium oxide solution exhibited a broad methylene region 5.2 ppm upfield from the aromatic protons, and a broad methyl triplet, which was 6.92 ppm upfield, with the correct ratio of 6:2:3 for combined aromatic-amide, methylene and methyl protons. The expected pattern for hydroxide adduct 7 was obtained from 2 in base, confirming the initial formation of 7 as an intermediate in the hydroxide catalyzed rearrangement of 2 to 3 and 4.

When 2 was allowed to rearrange in alcoholic sodium hydroxide, a mixture of 3 and 4 was obtained, in a ratio of about 1:2, with a trace of unrearranged 2 present. However, when 3 was treated similarly, no evidence of rearrangement to 2 or 4 was observed. The possibility of microscopic reversibility was further examined by the use of a trapping experiment. Sodium cyanide abstracts the C_2 proton from 3 to form the anion, as shown by the ultraviolet spectrum (Table 1, Column E), but if a reversible rearrangement occurs, then cyanide will add to the indenone intermediate, forming anion 8 in solution, and this anion can be trapped. A solution of 3 in dimethylsulfoxide was treated with a two-fold excess of sodium cyanide, and after eight hours the mixture was treated with methyl iodide. Work-up of the reaction gave a low yield of the methylated cyanide adduct 9, showing that there is a finite, though small, reversibility in the rearrangement of indenone 2 to 3.

Experimental

All melting points were determined in open capillary tubes with a Mel-Temp heating block and are corrected. Microanalyses were performed by Midwest Microlab, Inc., Indianapolis, IN. Infrared spectra were determined in potassium bromide disks with a Perkin-Elmer Model 137 Infracord, and were calibrated with polystyrene. Ultraviolet spectra were determined in 95% ethanol with a Bausch and Lomb Spectronic 505 Recording Spectrophotometer. Proton magnetic resonance spectra were obtained with a Varian A-60 spectrometer in indicated solvents using tetramethylsilane as an internal standard, except when hexadeuteriodimethyl-sulfoxide was the solvent, sodium2,2-dimethyl-2-silapentane 5-sulfonate (DDS) was used as the standard.

2-Carboxamido-3-ethyl-1-indenone (2) and 2-Carboxamido-3-ethylidene-1-indanone (3).

A solution of 2-cyano-3-ethylcinnamonitrile (1) (46 g, 0.252 mole) in 400 mL of concentrated sulfuric acid was heated on a steam bath for 30 min. The deep red solution was poured into 4 L. of ice; after 24 hr the solid was collected by filtration, washed thoroughly with water and dried to give 42 g (83%) of a mixture of indenone 2 and indanone 3. The two isomers were separated by the difference in their solubility in diethyl ether, using a Soxhlet apparatus. The yellow ether solution was concentrated to dryness at reduced pressure and the solid was recrystallized twice from 95% ethanol to give a pure sample of indenone 2 (5.06 g, 10%) as evidenced by tlc on silica gel using benzene:acetic acid (50:1), which exhibited one spot, R_f 0.06: mp 147-149°, solidified and turned white and remelted 190-192° (lit (1) 148-150°); ir 2.95 and 3.18 (NH_2), 5.90 (CO), 6.00 ($CONH_2$), and 6.20-6.35 μ (aromatic); uv (λ max) 248 (ϵ 40,800) and 254 mμ (ϵ 40,700), originally reported (1) 244 (ϵ 36,300) and 249 mμ (ϵ 35,300); pmr ($CDCl_3$) δ 7.75 (1H, broad singlet, amide), 7.37 (4H, multiplet, aromatic), 6.10 (1H, broad singlet, amide), 3.21 (2H, quartet, J = 7.5 cps, methylene), and 1.31 (3H, triplet, J = 7.5 cps, methyl).

The solid remaining in the Soxhlet cup was rinsed with ether to remove the last traces of 2 and recrystallized twice from 95% ethanol to give 11.95 g (22%) of indanone 3 as evidenced by tlc on silica gel using benzene:acetic acid (50:1), which exhibited one spot, R_f 0.14: mp 197-199° (lit (3) 199-200°); ir 2.97 and 3.12 (NH_2), 5.82 (CO), 6.06 ($CONH_2$), and 6.25 μ (aromatic); uv (λ max) 242 (ϵ 32,200), 264 (ϵ 17,000), 271-274 (ϵ 14,200), and broad band centered at 330 mμ (ϵ 2,250); pmr (DMSO-d_6) δ 7.6 (6H, complex multiplet, aromatic and amide), 6.54 (1H, quartet, J = 7 cps, further split into 1 cps doublets by long range coupling, vinyl), 4.21 (1H, broad singlet long range coupling, C_2H), and 1.88 (3H, couplet, J = 7 cps, methyl).

Z-2-Carboxamido-2-methyl-3-ethylidene-1-indanone (5).

Sodium hydride (0.27 g of 50% NaH in mineral oil, 5.4 mmoles) was added to a flask, fitted with a drying tube (Drierite), containing 1.08 g (5.0 mmoles) of indenone 2 in 25 mL of dry tetrahydrofuran. After stirring at room temperature for 4 hr methyl iodide (2.5 mL) was added and the reaction was maintained at room temperature for 55 hr. The reaction mixture was diluted with 200 mL of water, the excess methyl iodide was removed on a steam bath, and the solution extracted twice with 250 mL portions of chloroform. The chloroform solution was dried (magnesium sulfate), decolorized (Norit), and evaporated to dryness at reduced pressure. The residue was triturated with cyclohexane and collected to give 5 (0.42 g, 39%). An analytical sample of white plates was prepared by recrystallization from benzene: mp 180-182°; ir 2.90 and 3.14 (NH_2), 5.84 (CO), 6.01 ($CONH_2$), 6.24 (aromatic), and 13.0 μ (alkene); uv (λ max) 241 (ϵ 31,700), 265 (ϵ 16,500), 271-274 (ϵ 14,800) and 333 mμ (ϵ 3,100); pmr (DMSO-d_6) δ 7.5 (6H, complex multiplet, aromatic and amide), 6.00 (1H, quartet, J = 7 cps, vinyl), 2.14 (3H, doublet, J = 7 cps, methyl), and 1.42 (3H, singlet, methyl).

Anal. Calcd for $C_{13}H_{13}NO_2$: C, 72.56; H, 6.05; N, 6.51. Found: C, 72.35; H, 6.19; N, 6.46.

E-2-Carboxamido-2-methyl-3-ethylidene-1-indanone (6).

Compound 6 was synthesized in 50% yield from indanone 3 following the procedure described above for 5. An analytical sample of white plates was prepared by recrystallization from benzene: mp 142-143.5°; ir 2.91 and 3.18 (NH_2), 5.85 (CO), 5.98 ($CONH_2$), 6.25 (aromatic), and 13.3 μ (alkene); uv (λ max) 241 (ϵ 29,000), 265 (ϵ 16,100) 271-274 (ϵ 13,400), and 334 mμ (ϵ 2,400); pmr (DMSO-d_6) δ 7.5 (6H, complex multiplet,

aromatic and amide), 6.56 (1H, quartet, J = 7.5 cps, vinyl), 1.89 (3H, doublet, J = 7.5 cps, methyl), and 1.43 (3H, singlet, methyl).

Anal. Calcd for $C_{13}H_{13}NO_2$: C, 72.56; H, 6.05; N, 6.51. Found: C, 72.43; H, 6.23; N, 6.50.

Isomerization of Indenone 2 with Sodium Hydroxide to Indanones 3 and 4.

Indenone 2 (1 g, 5 mmoles) was added to a solution of sodium hydroxide (0.2 g, 5 mmoles) to 50 mL of 95% ethanol and 2 mL of water. The solution was maintained at room temperature for 120 hr, diluted with 150 ml of water and acidified to pH 3 with 10% hydrochloric acide. The off-white precipitate was collected by filtration to give 0.45 g of 4 (45%). Recrystallization from 95% ethanol and washing with diethyl ether afforded an analytical sample of white powder, which showed one spot of tlc (benzene:acetic acid, 50:1), R_f 0.14: mp 129-130°, the sample turned yellow, solidified, remelted 145°, turned white, solidified and melted again at 190°; ir 2.95 and 3.12 (NH_2), 5.85 (CO), 6.01 ($CONH_2$), and 6.15-6.30 μ (aromatic); uv (λ max) 242 (ϵ 31,500), 255 inflection (ϵ 19,000), 261-264 (ϵ 15,800), 271-274 (ϵ 12,500), and 326 mμ (ϵ 4,200); pmr (DMSO-d_6) δ 7.5 (complex aromatic), 6.04 (quartet, J = 8 cps further split into 2 cps doublets, vinyl), 4.17 (broad singlet, C_2H), and 2.14 (doublet, J = 8 cps further split into 2cps doublets, methyl). The compound, after solution in DMSO, turned slightly yellow indicating isomerization to 2, and minor peaks were observed at δ 1.31 and 3.21 for the methyl and methylene protons of 2.

Anal. Calcd for $C_{12}H_{11}NO_2$: C, 71.64; H, 5.47. Found, C, 71.58; H, 5.69.

The mother liquor was extracted with three 200 mL portions of chloroform; the chloroform solution was dried with magnesium sulfate, and evaporated to dryness at reduced pressure. The residue was recrystallized from 95% ethanol to give 0.2 g (20%) of indanone 3 contaminated with a trace of 2 as evidenced by tlc (benzene:acetic acid, 50:1), major spot R_f 0.14 and minor spot R_f 0.06. The yellow indenone 2 was removed by treating the solid with diethyl ether to give pure 3 as evidenced by congruent infrared spectrum and melting point. The isomerization was also carried out using a five-fold excess of sodium hydroxide to give 37% of 4 and 30% of 3.

Treatment of 3 with Sodium Hydroxide.

Indanone 3 was added to a solution of 50 mL of 95% ethanol and 50 mL of 10% sodium hydroxide. After 100 hr at room temperature the solution was diluted with 200 mL of water and acidified to pH 2 with sulfuric acid. The resulting solid (40%) was collected and proved to be 3 by congruent infrared spectrum and melting point. The mother liquor was extracted with chloroform, and the chloroform phase was dried (magnesium sulfate), concentrated to dryness at reduced pressure, and the solid was triturated with hexane, and collected to give an additional 45% of 3 as evidenced by congruent infrared spectrum and melting point.

2-Carboxamido-2-methyl-3-cyano-3-ethyl-1-indanone (9).

A solution of 3 (2 g, 10 mmoles) and sodium cyanide (1g, 20 mmoles) in 20 mL of dimethyl sulfoxide was stirred at room temperature for 8.5 hr and then methyl iodide (5 mL) was added. After an additional 24 hr the solution was diluted with 250 mL of water and the excess methyl iodide was removed on a steam bath. The cooled solution precipitated 0.46 g (20%) of 9. The mother liquor was extracted with chloroform, but work-up of the chloroform solution only produced an oil which resisted further purification. Analytical sample of 9 (white plates) was prepared by recrystallization from 50% ethanol: mp 197-199°; ir 2.90 and 3.10 (NH_2), 3.35 (CH), 4.44 (CN), 5.83 (CO), 5.93 ($CONH_2$), and 6.30 μ (aromatic).

Anal. Calcd for $C_{14}H_{14}N_2O_2$: C, 69.42; H, 5.79; N, 11.58. Found: C, 69.52; H, 6.02; N, 11.50.

Ultraviolet Studies. A 2.0×10^{-5} M solution of 2 or 3 in 95% ethanol was prepared by dissolving 4 mg of either 2 or 3 in 100 mL of 95% ethanol and diluting 5 mL of this solution to a volume of 50 mL. The solutions of 2 and 3 in base were prepared by adding 10 drops of 10% sodium hydroxide (ca. 2.5×10^{-2} M) to a 2.0×10^{-5} M solution of 2 or 3 in 50 mL of 95% ethanol. The solutions for the sodium cyanide spectra were prepared by dissolving 5 mg of 2 or 3 and 10 mg of sodium cyanide in 100 mL of 95% ethanol and diluting 5 mL of this solution to a volume of 50 mL to give solutions that were 2.5×10^{-5} M in indenone or indanone and 2.0×10^{-4} M in sodium cyanide. The acidified spectra were run after adding enough 1N hydrochloric acid to the cell to lower the pH to 1. All spectra were run against the appropriate blank on a Bausch and Lomb 505 spectrophotometer.

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Note

[1] Taken in part from a thesis submitted to Indiana University by D.A.T. in partial fulfillment of the requirements for the degree of Doctor of Philosophy, September, 1968.

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NMR Spectroscopic and Theoretical Investigation of the Bonding in $W_3(OR)_9(\mu_3\text{-}CCH_3)$ and Its Comparison to $Co_3(CO)_9(\mu_3\text{-}CCH_3)$.

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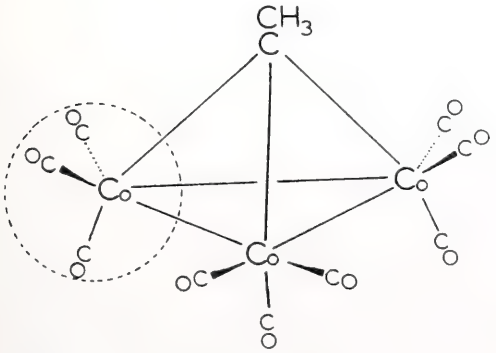
Introduction

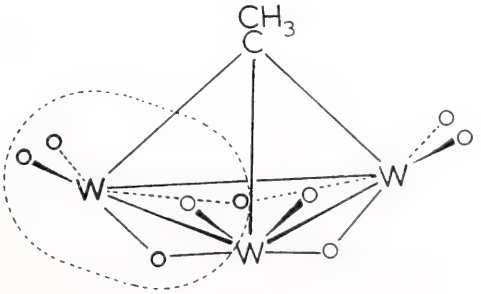
Metal clusters have in the last number of years been proposed to be models for the study of reactivity on metal surfaces. This proposed modeling of surfaces has been the driving force behind much synthetic and theoretical organometallic chemistry. Among the oldest, and most thoroughly studied organometallic clusters are the alkylidyne capped tricobalt nonacarbonyls. Of particular interest with respect to such alkylidyne capped trinuclear clusters is the nature of the bond between the metal cluster fragment, $M_3L_9^{3+}$, and the small organic fragment, the alkylidyne. Theoretical studies (1) and the short C-C single bond distance (1.37 Å) from the crystal structure of $[(CO)_9Co_3C]_2$, (2) indicative of a butadiyne type conjugation of π systems, suggest that the apical carbon of the $Co_3(CO)_9(\mu_3\text{-}CR)$ clusters is approximately sp hybridized. While there is not yet such conclusive experimental evidence for the early transition metal complexes, $W_3(OR)_9(\mu_3\text{-}CR)$, alkyl like $^{13}C\text{-}^{13}C$ and $^{185}W\text{-}^{13}C$ one bond coupling constants, typical of an sp^3 hybridized apical carbon, suggest that some variation exists in the mode of alkylidyne to metal cluster bonding between clusters of early and late transition metals. Fenske-Hall Molecular Orbital calculations of $Co_3(CO)_9(\mu_3\text{-}CCH_3)$ and $W_3(OH)_9(\mu_3\text{-}CCH_3)$ support this experimental trend. Beyond possible insight into the binding of small organic fragments to metal surfaces, an understanding of such a bonding variation between clusters of early and late transition metals may elucidate a range or limits to the isolobal analogy between organometallic and organic fragments.

Results and Discussion

The alkylidyne capped tricobalt nonacarbonyl and tritungsten nonaalkoxide clusters are electronic and structural analogues. Electronically the metals Co^{+1} and W^{+4} may be related by a $d^8\text{-}d^2$ electron/hole formalism. Structurally the tungsten and cobalt clusters are related by their M_3C core. The arrangement of the supporting ligands, however, is different in each cluster. The nine carbonyl ligands of the cobalt cluster are terminally bound in such a way as to give the local pseudo-octahedral geometry about each cobalt seen in structure A. Thus one may visualize the $Co_3(CO)_9(\mu_3\text{-}CR)$ clusters as the assemblage of three ML_3 fragments capped by a triply bridging alkylidyne. By contrast three of the



A



B

nine alkoxide ligands in the tungsten cluster bridge each of the three metal-metal bonds, the other six are terminal. This results in the pseudo-square based pyramidal geometry about each tungsten seen in structure B. A division of each bridging alkoxide allows the tungsten cluster to be visualized as the assemblage of three ML_4 fragments, capped by a triply bridging alkylidyne ligand.

The molecular orbital interaction diagram of the $Co_3(CO)_9^{3+}$ and CCH_3^{3-} fragments is shown in Figure 1. On the left are the fragment orbitals resulting from the assemblage

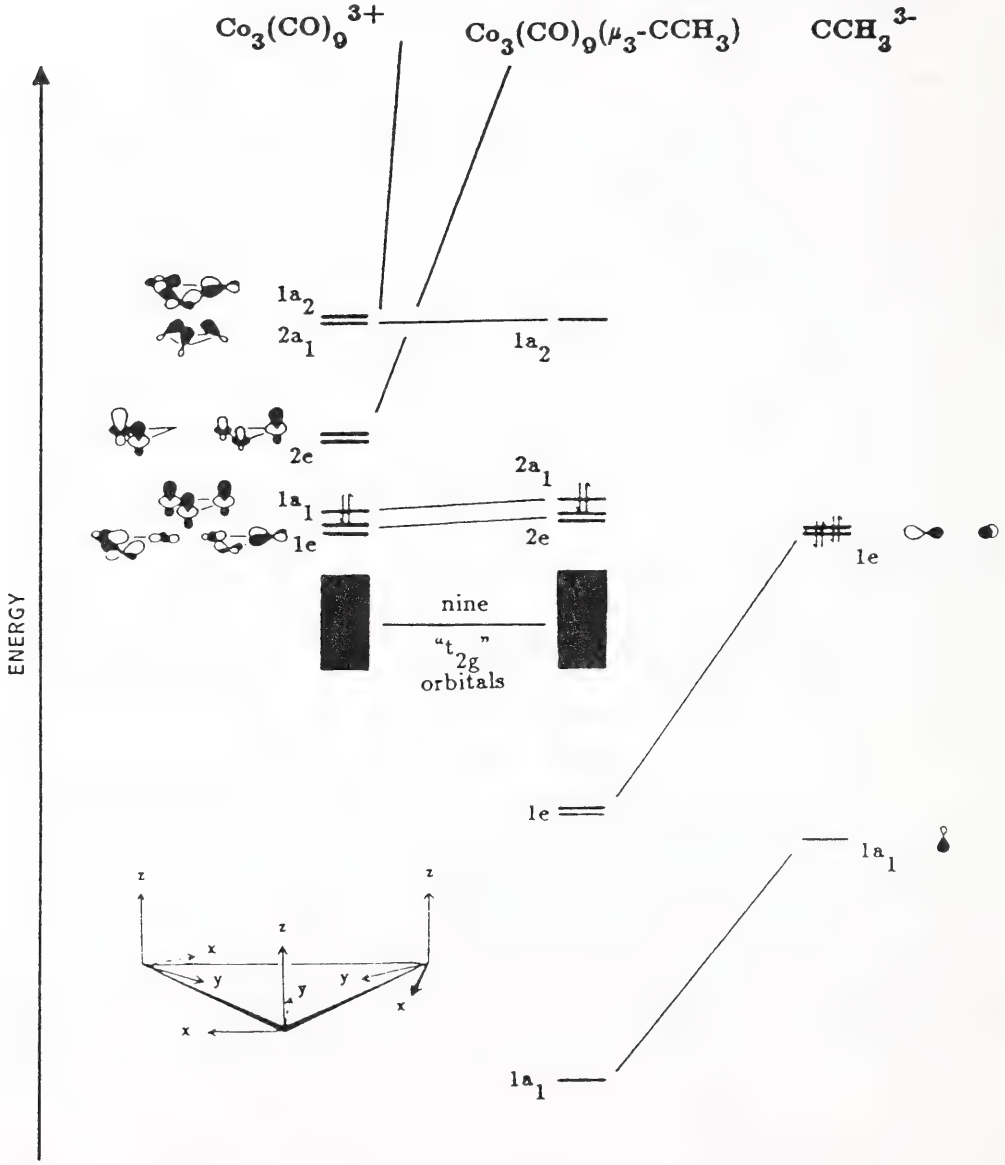


FIGURE 1: Interaction diagram for $Co_3(CO)_9(\mu_3\text{-}CCH_3)$.

of three ML_3 fragments. Of lowest energy are the nine pseudo-t_{2g} orbitals derived from linear combinations of the non-bonding t_2 orbitals of the ML_3 fragment. These orbitals have been stabilized by π-back bonding to the CO π^* oribtals, but undergo no further interaction with the cluster. The la_1 and le metal cluster fragment orbitals, formally derived from metal-ligand σ-antibonding orbitals, form the metal-metal bonds leaving the 2e, formally metal-metal and metal-ligand σ-antibonding, and the high lying $2a_1$, an spd hybrid, to interact with the incoming alkylidyne. This interaction is insufficient to in-

duce a rehybridization of the alkylidyne fragment oribtals. Instead the la_1 sp-like hybrid of the alkylidyne is delocalized between all three metal atoms (1).

In contrast, both the ligand field and electron count of the tungsten cluster present a considerably different bonding picture, described in the interaction diagram of Figure 2. The important $W_3(OR)_9^{3+}$ fragment orbitals are pictured to the left of the interaction

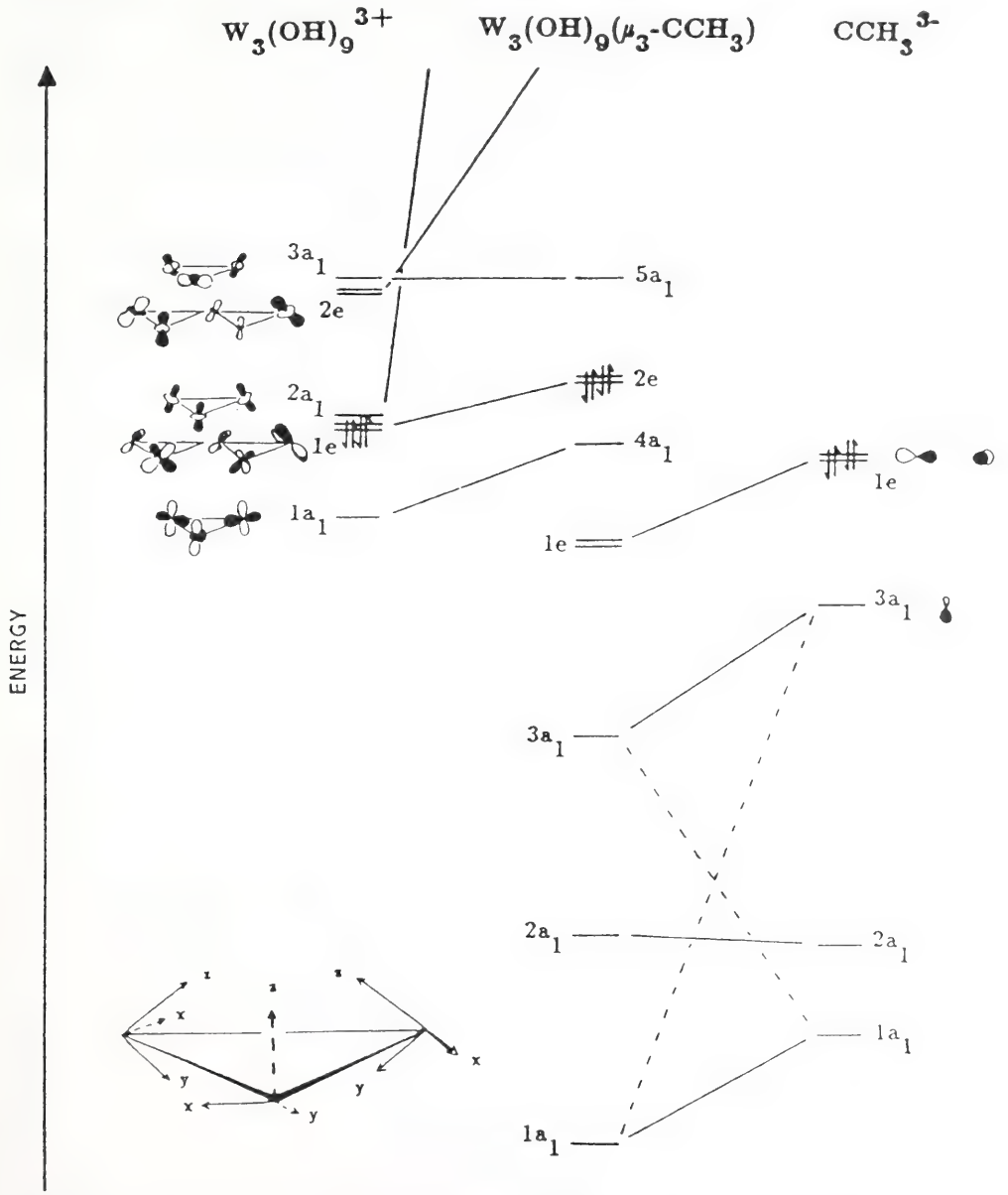


FIGURE 2: Interaction diagram for $W_3(OH)_9(\mu_3\text{-}CCH_3)$.

diagram. Not represented are the higher lying metal based orbitals, destabilized by π donation of the alkoxide ligands, and the highest metal-ligand antibonding orbitals. The $1a_1$, $2a_1$, and 1e cluster fragment orbitals, with six electrons, form the metal-metal bonds. These as well as the slightly higher 2e orbitals have been derived from linear combinations of the formally non-bonding orbitals of the ML_4 fragments, providing a more favorable energy match with the alkylidyne fragment than was provided by the antibonding oribtals of the cobalt fragment. The net result of this interaction is a severe rehybridiza-

tion of the sp-like $3a_1$ alkylidyne fragment orbital such that in the final $W_3(OR)_9(\mu_3\text{-}CCH_3)$ cluster the C2s character of the former sp-like hybrid is now in the $1a_1$ cluster orbital, leaving the alkylidyne component of the $3a_1$ cluster orbital to be 99.9% $C2p_z$.

This separation of the C2s and $C2p_z$ character is reminiscent of the molecular orbital description of the classical sp^3-hybrid, methane. When viewed from the perspective of C_{3v} symmetry, both a classical sp and an sp^3 hybridized carbon should have two molecular orbitals of a_1 symmetry and one set of orbitals of e symmetry. Both a_1 orbitals of the sp hybrid should contain an approximately 50:50 mixture of the C2s and $C2p_z$, whereas the sp^3 hybrid should have the C2s character localized in the $1a_1$ orbital and the $C2p_z$ character localized in the $2a_1$ orbital. Thus we suggest that the apical carbon of $Co_3(CO)_9(\mu_3\text{-}CCH_3)$ is hybridized in an sp-like fashion while the apical carbon of $W_3(OR)_9(\mu_3\text{-}CCH_3)$ is hybridized in an sp^3-like manner.

We had hoped to make use of the correlation established for organic systems (3) between the hybridization of carbon atoms and their one bond coupling constants. Unfortunately the ethylidyne one bond carbon-carbon coupling constants of both clusters were found to be virtually identical, and of the magnitude expected for an sp^3-sp^3 interaction (see Table 1). Furthermore the ethylidyne coupling constant ($^1J_{^{13}C-^{13}C}$) of the

Table 1. A Comparison of $^1J_{^{13}C-^1H}$, $^1J_{^{13}C-^{13}C}$, $^1J_{^{183}W-^{13}C}$, and the Total C2s-C2s Overlap Population.

	$^1J_{^{13}C-^1H}$	$^1J_{^{183}W-^{13}C}$	$^1J_{^{13}C-^{13}C}$	C2s-C2s O.P.
$W_3(O^iPr)_9(\mu_3\text{-}CCH_3)$	126.4	136.9	36.1	0.141
$(^tBuO)_3W\equiv CCH_3$	126.8	302.1	32.2	0.129
$Co_3(CO)_9(\mu_3\text{-}CCH_3)$	127.7	—	31.2	0.119
$HC\equiv CCH_3$	131	—	67.4	—
$HC\equiv CH$	248.7	—	170.6	—
$(^tBuO)_3W\equiv CH$	150	289	—	—

tungsten alkylidyne monomer, $(^tBuO)_3W\equiv CCH_3$, is 32.2 Hz rather than the approximately 60 Hz coupling expected for an sp-sp^3 interaction as seen in $HC\equiv CCH_3$. This suggests that a one bond carbon-carbon coupling constant analysis is not a valid measure of the hybridization of a metal bound carbon. Nonetheless, the direct correlation between the one bond carbon-carbon coupling constants and the calculated total C2s-C2s overlap population suggests that one can legitimately infer the polarization of the C2s orbital from the coupling constants.

Conclusion

While the one bond carbon-carbon coupling constants have been shown to be an ineffective probe into the bonding between alkylidyne and trinuclear metal fragments, Fenske-Hall Molecular Orbital calculations have shown a variation to exist between the bonding of the $Co_3(CO)_9(\mu_3\text{-}CCH_3)$ and $W_3(OR)_9(\mu_3\text{-}CCH_3)$ clusters. Current experimental work is anticipated to confirm this trend. The significance of this bonding variation is best explained by the contrasting isolobal analogies presented by these two clusters (Figure 3). With an sp-like hybridization of its apical carbon the alkylidyne tricobalt nonacarbonyl cluster is isolobal to acetylene, thus the $Co_3(CO)_9^{3+}$ fragment may be considered isolobal to CH^{3+}. By contrast the alkylidyne tritungsten nonaalkoxide cluster, with an sp^3-like hybridization of its apical carbon, is isolobal to a carbon tetrahedrane, C_4H_4, and the $W_3(OR)_9^{3+}$ fragment is isolobal to the π orbitals of $C_3H_3^{3+}$.

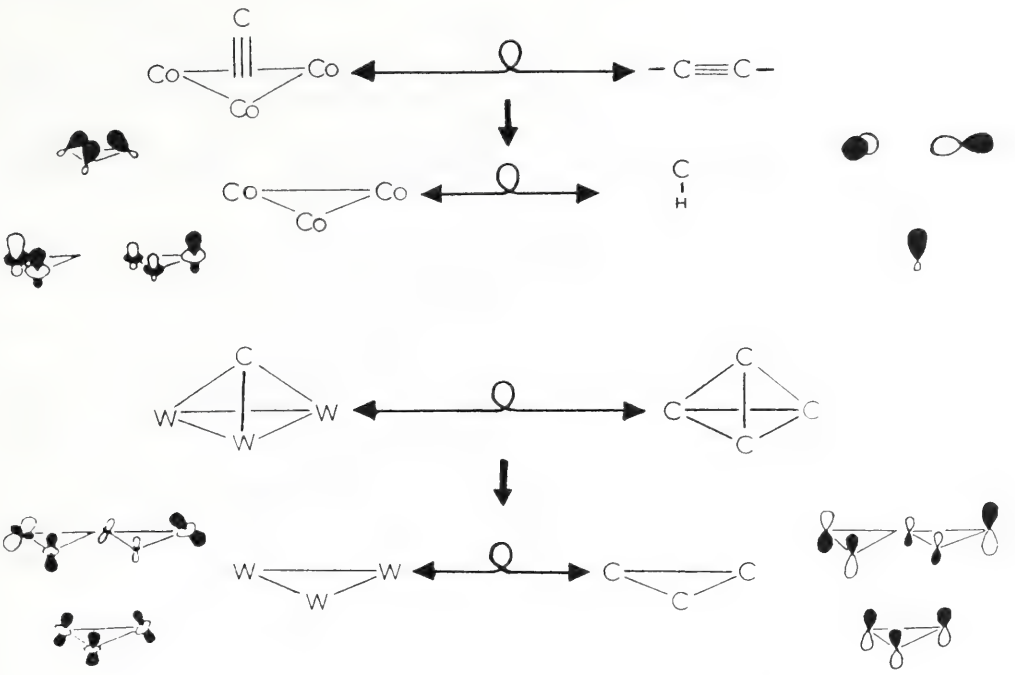


FIGURE 3: The contrasting isolobal analogies of $Co_3(CO)_9(\mu_3\text{-}CCH_3)$ and $W_3(OH)_9(\mu_3\text{-}CCH_3)$.

It is predicted that such contrasting limits of many such isolobal analogies may be articulated by the comparison of transition metal complexes of early and late transition metals.

Experimental

The coordinates for the model compounds $Co_3(CO)_9(\mu_3\text{-}CCH_3)$ and $W_3(OH)_9(\mu_3\text{-}CCH_3)$ were taken from the crystal structures of $Co_3(CO)_9(\mu_3\text{-}CCH_3)$ (4) and $W_3(O^iPr)_9(\mu_3\text{-}CCH_3)$ (5) and idealized to C_{3v} symmetry.

Molecular orbital calculations were performed using the Fenske-Hall method described elsewhere (6). All calculations were performed using the VAX 11/780 computer at the Indiana University Computational Chemistry Center. Atomic basis functions were generated by a best fit to Herman Skillman atomic calculations (7). Contracted double-ζ representations were used for Co 3d and W 5d AO's as well as C and O 2p AO's. Basis functions for the metal atoms were generated for a +1 oxidation state with valence s and p exponents fixed at 2.00 for Co 4s and 4p, and 1.80 for W 6s and 6p.

All ^1H NMR spectra were recorded in benzene-d_6 or toluene-d_8 on a Nicolet-360 spectrometer at 360 MHz. ^{13}C NMR spectra of the tungsten complexes were recorded in benzene-d_6 or toluene-d_8 on a Varian XL-300 spectrometer at 75 MHz. ^{13}C NMR spectra of $Co_3(CO)_9(CCH_3)$ was recorded in C_6D_{12} on a modified Nicolet-200 spectrometer at 50.3 MHz.

Acknowledgment

We would like to thank Professor Adam Allerhand and Steve Maple for determining the ethylidyne coupling constant of $Co_3(CO)_9(\mu_3\text{-}CCH_3)$, and Professor Odile Eisenstein for many helpful discussions.

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Heavier Main-group Atoms as Ligands to Transition-metal Centers

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Introduction

Heavy main-group elements of the third and lower rows are distinguished from their lighter congeners by the presence of filled p-orbitals in their cores. The greater radial extension of heavy element np vs. ns valence orbitals results in greater p character for σ bonds formed by them compared to σ bonds formed by the n = 2 elements. Consequently, second-row elements exhibit striking differences in their chemistry when compared to the heavier members of their families. This is well known for simple inorganic and organo main-group compounds, and the results summarized below serve to illustrate this fact for main-group transition-metal chemistry.

Results and Discussion

Compounds of the type $M(O)(OR)_4$, where M = Mo or W and R = a variety of alkyl groups, have been known for over 25 years (3,5). Recent work has shown that they undergo comproportionation reactions with $M_2(OR)_6$ species containing a metal-metal triple bond, forming triangular clusters of the type $M_3(\mu_3\text{-}O)\ (\mu_3\text{-}OR)\ (\mu\text{-}OR)_3(OR)_6$ (4). The electrochemistry and UV-visible spectra of these latter compounds have been correlated with their electronic structure based on Fenske-Hall molecular orbital calculations (2). In order to compare differences in physical properties caused by small perturbations in the ligand environment surrounding these metal clusters, it was of interest to synthesize the analogous sulfido-capped metal triangles.

The compounds $W(S)(OR)_4$, where R = tert-butyl or isopropyl, are made by adding $WSCl_4$ to the appropiate lithium alkoxide (4 equiv) in ether at $-78°$. ^1H NMR spectroscopic data revealed only one type of alkoxide ligand for each compound, suggesting that both are monomeric in solution. An X-ray crystal structure determination confirmed this for the solid-state structure of $W(S)(OBu^t)_4$ (Figure 1). However, all attempts at comproportionation reactions between these compounds and $W_2(OR)_6$ (R = Bu^t, Pr^i, or CH_2Bu^t) species failed to yield any evidence for the desired $W_3(\mu_3\text{-}S)(\mu_3\text{-}OR)(\mu\text{-}OR)_3(OR)_6$ product. Space-filling diagrams of the $M_3(\mu_3\text{-}O)$ species suggest that the pocket for the μ_3-E ligand formed by the surrounding alkoxides is simply too small to accommodate a sulfur atom with its van der Waals radius *ca.* 0.45 Å larger than that of oxygen, the difference in radii being attributable to the ten core electrons of sulfur.

In 1982 Schrock *et al.* reported the preparation of $[(Bu^tO)_3W\equiv N]_x$ by metathesis of $W_2(OBu^t)_6$ with acetonitrile or benzonitrile (8). Alkoxide-for-halide ligand exchange on $Mo(N)Cl_3$ gives the corresponding molybdenum compound (1). X-ray crystal structure determinations revealed that both nitrido compounds exist in the solid state as linear polymers of trigonal pyramidal $(RO)_3M\equiv N$ units bound head-to-tail via weak M---N bonds. While many other transition-metal compounds with terminal nitrido ligands are known, there are as yet no structurally-characterized examples of terminal phosphides. With this in mind, we investigated the reactivity of P_4 with $M_2(OR)_6$ (M = Mo, W; R = Bu^t, Pr^i, CH_2Bu^t) species.

The reaction between $W_2(OCH_2Bu^t)_6(HNMe_2)_2$ and P_4 in toluene at 75° for four hours gives two products. The product formed in 75% yield has two types of neopentoxide ligands in a 2:1 ratio and contains no phosphorus according to elemental analysis

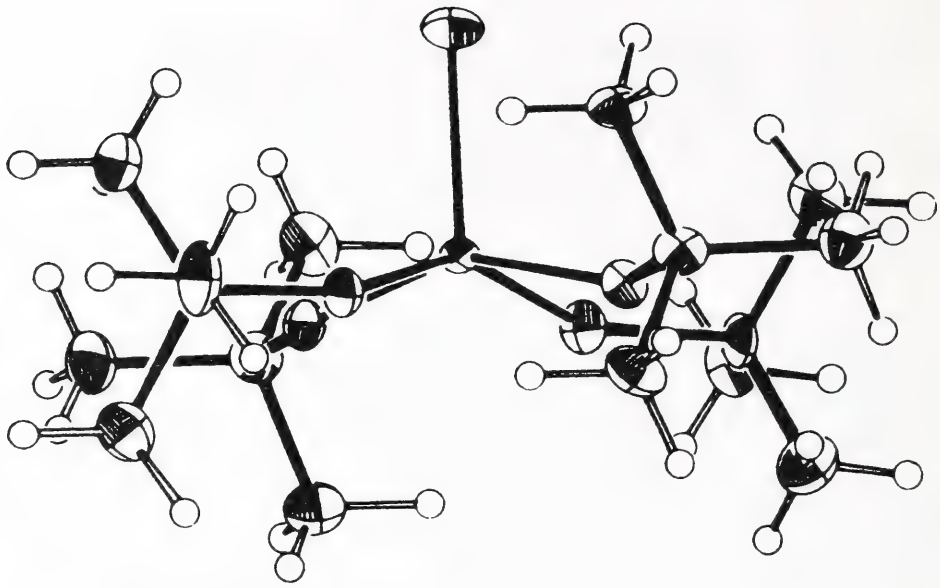

FIGURE 1. ORTEP diagram of $W(S)(OBu^t)_4$. Pertinent interatomic distances (Å) and angles (°) are: W-S, 2.1396(13); W-O, 1.886(3); S-W-O, 105.09(10); W-O-C, 142.47(27); O-W-O(cis), 86.13(13); O-W-O-(trans), 149.83(13).

and ^{31}P NMR spectroscopy. It is the subject of continuing study. The other product, formed in 25% yield, has only one type of neopentoxide ligand and displays a ^{31}P NMR resonance upfield at -205.2 ppm vs. 85% H_3PO_4 with a small coupling to ^{183}W of 16 Hz. An X-ray crystal structure determination revealed it to be $(\eta^3\text{-}P_3)W(OCH_2Bu^t)_3(HNMe_2)$ (Figure 2). This represents the first member in a class of

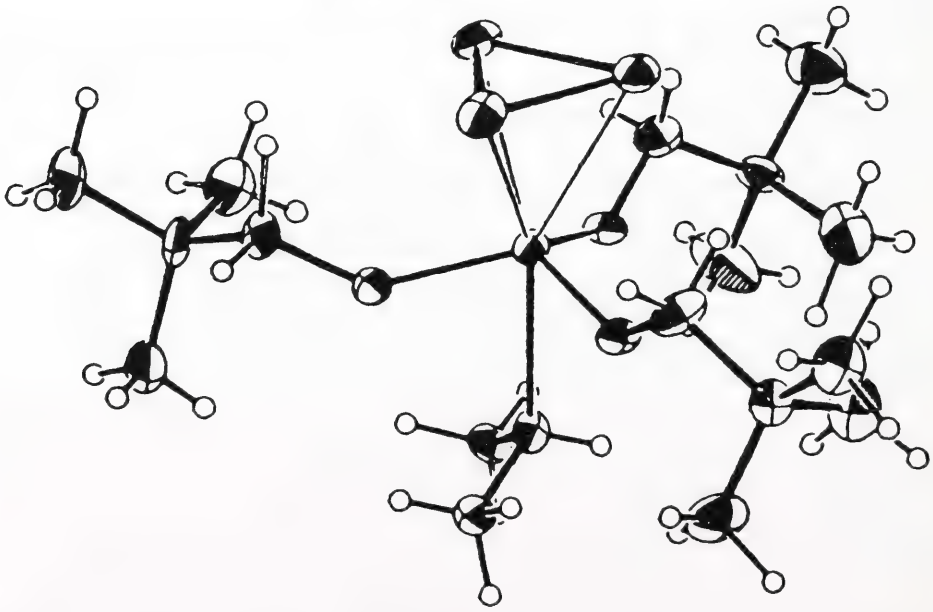

FIGURE 2. ORTEP diagram of $(\eta^3\text{-}P_3)W(OCH_2Bu^t)_3(HNMe_2)$. Pertinent interatomic distances (Å) and angles (°) are: W-P, 2.4930(25); W-O, 1.908(5); W-N, 2.315(7); P-P, 2.155(4); P-W-P, 51.23(9); P-W-O(proximal), 88.34(17); P-W-O(distal), 134.15(17); W-O-C, 136.6(5); P-W-N, 150.03(18); O-W-O, 114.17(24); O-W-N, 75.80(24); W-P-P, 64.39(9).

P_3-containing monomeric compounds that is supported by π-donating ligands, all of which used P_4 as the source of the P_3 fragment. Notable by their absence are any transition-metal compounds containing a terminal phosphide ligand or an η-N_3 ligand. This can be attributed to the comparable radial extensions of the 2s and 2p orbitals, and the greater radial extension of the 3p vs. the 3s orbital (6). In the η^3-N_3 case the similar orbital radii would facilitate sp hybridization which, together with the large N_2 bond strength, would cause ligand disproportionation to a terminal M≡N species and N_2. In the M≡P case the phosphorus 3p π orbitals would be rather diffuse and oriented orthogonally to the metal-phosphorus vector, thus giving poor M-P π overlap and consequently weak M-P π bonds, while the M-P σ bond would be expected to have little phosphorus s character. In a Fenske-Hall molecular orbital calculation on the model compound $(\eta^3\text{-}P_3)W(OH)_3(NH_3)$ both the σ and π components of the W-to-P_3 bond are predicted to arise from phosphorus 3p orbitals oriented parallel to the metal-phosphorus vector. This results in much greater metal-phosphorus overlap and thus a much stronger bond. The phosphorus 3s orbitals remain essentially unhybridized and are used to form P-P σ bonds.

The amide ligand is pervasive in inorganic and organometallic chemistry. The synthesis, chemical, and physical properties of compounds containing it have been exhaustively reviewed (7). However, its heavier group 15 analogues are comparatively scarce. Since arsenic forms weaker bonds to transition metals and carbon than does nitrogen, prospects for novel chemistry of the arsenide ligand looked promising.

Reaction of $1,2\text{-}M_2Cl_2(NMe_2)_4$ (M = Mo, W) with 2 equivalents of $LiAsBu^t_2$ in ether at -78° in the dark yields $1,2\text{-}M_2(AsBu^t_2)_2(NMe_2)_4$. X-ray crystal structure determinations revealed that both compounds crystallized in the anti rotameric form and are isostructural (Figure 3). In comparing the amide and arsenide ligands, the former's short bond lengths to the metal atoms and their planarity argue strongly for sp^2 hybridization of the nitrogen atoms with a consequently large amount of N → M π donation. In contrast, the long M-As distances, which are comparable to what one would expect based

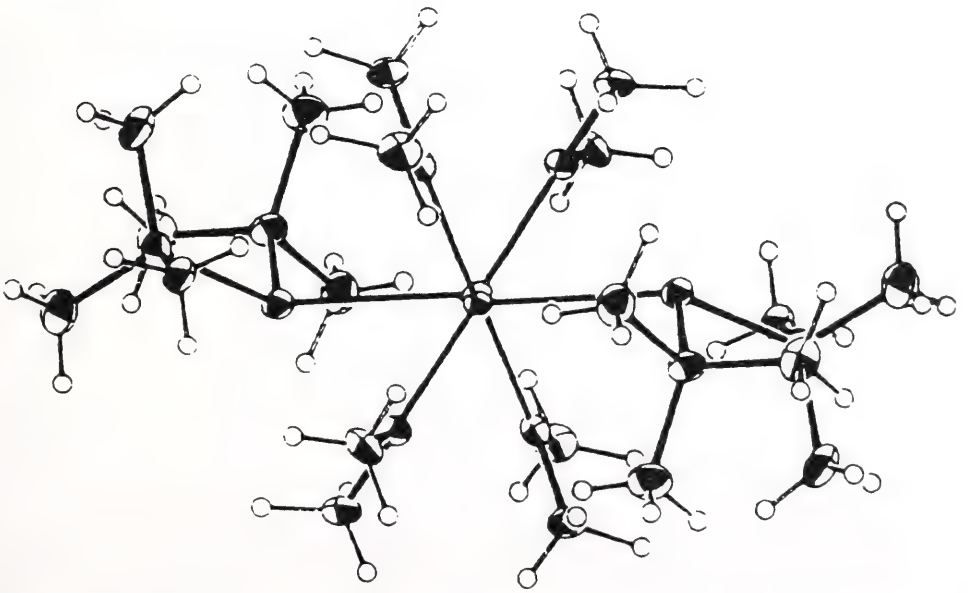

FIGURE 3. ORTEP diagram of $1,2\text{-}Mo_2(AsBu^t_2)_2(NMe_2)_4$ viewed down the metal-metal vector. Pertinent interatomic distances (Å) are: Mo-Mo, 2.2159(12); Mo-N, 1.972(6); Mo-As, 2.6161(11). For the tungsten analogue: W-W, 2.3001(11); W-N, 1.955(9); W-As, 2.5949(15).

on the sums of covalent radii, and the pyramidality about the arsenic atoms both suggest that the arsenic atoms are only singly bonded to the metal atoms using orbitals that have predominantly p character. Variable-temperature ^{1}H NMR spectroscopy further revealed that, while rotation about the M-N bonds was rapid at room temperature on the NMR time scale, M-As bond rotation and arsenic inversion were also rapid and had lower activation barriers than M-N bond rotation. In fact, while both bond rotation processes were frozen out in the gauche rotamers by -85 °, inversion at arsenic was still rapid. This clearly contradicts the expectation that inversion at heavier group 15 atoms should be much slower than at nitrogen, but is consistent with a transition state having some degree of As → M π bonding stabilized by the empty acceptor orbitals on the metal atoms.

Conclusion

The examples given above illustrate the fact that, while heavier main-group elements share common oxidation states with their second row family members, their behavior otherwise can be radically different. This should not be unexpected, however, considering the differences between the 3d and lower transition metals, or between the lanthanides and actinides. Perhaps all this really says is that, although trends can be discerned in the general properties of the elements, each one is still unique and we shouldn't be surprised when they behave as such under closer scrutiny.

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Carbon-Nitrogen Bond Formation in the Reactions of Unsaturated Molecules with $Mo(NMe_2)_4$

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Introduction

In 1971, Chisholm and Bradley published the synthesis and characterization of $Mo(NMe_2)_4$, an air and moisture sensitive compound.(2) The molecule contains four amido ligands distributed around molybdenum as pictured in Figure 1.(4). The MoN_4 and

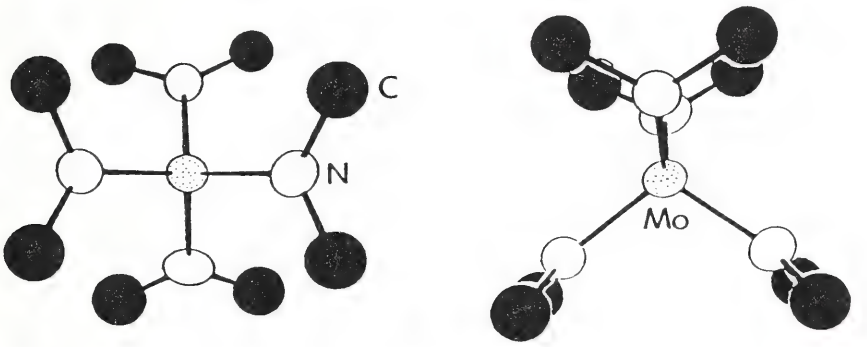

Figure 1. Two ORTEP drawings of $Mo(NMe_2)_4$ with hydrogen atoms omitted.

$Mo(NC_2)_4$ units have virtual T_d and D_{2d} symmetry, respectively. All the amido ligands are planar and the Mo-N distances are short, 1.93 Å, indicative of π-bonding to the metal center which achieves a formal 18 valence electron configuration. In D_{2d} symmetry the two d-electrons are paired and result in a monomeric Mo(IV) diamagnetic compound.

There are numerous molecules which have demonstrated the ability to insert into the M-N bond of a metal amide, for example: $C{\equiv}O$, $C{\equiv}NR$, CO_2, CS_2, $RC{\equiv}N$, $M_2(CO)_n$, $RN{=}O$, $RC{\equiv}CR'$, $R_2C{=}CR_2$.(7) This study was undertaken to explore the chemistry of $Mo(NMe_2)_4$ to gain fundamental insight into the reactivity of high oxidation state molybdenum species supported by strong π-bonding amido ligands towards various small unsaturated molecules. All reactions were carried out under inert atmospheres and all products are air and moisture sensitive.

Results and Discussion

Carbon Monoxide Reactions

The intense purple color of hydrocarbon solutions containing $Mo(NMe_2)_4$ changes instantaneously to brown upon exposure to carbon monoxide (>4 equiv). The ^1H and ^{13}C NMR spectra show the major diamagnetic product formed is 1,1,3,3-tetramethylurea which is consistent with a reaction pathway involving (i) $C{\equiv}O$ insertion to give a $Mo[C(O)NMe_2]$ moiety and (ii) reductive elimination by C-N bond formation: "$Mo(IV)[C(O(NMe_2](NMe_2)$" → "Mo(II)" + $(Me_2N)_2C{=}O$. A heterogeneous brown solution results when pentane is used as the solvent. Filtration of the insoluble powder and reduction of the volume of the filtrate yields a pure crystalline metal-containing com-

pound, $Mo_3[C(O)NMe_2]_6(CO)_6(HNMe_2)_2$. A single crystal X-ray study revealed the molecular structure shown in Figure 2.

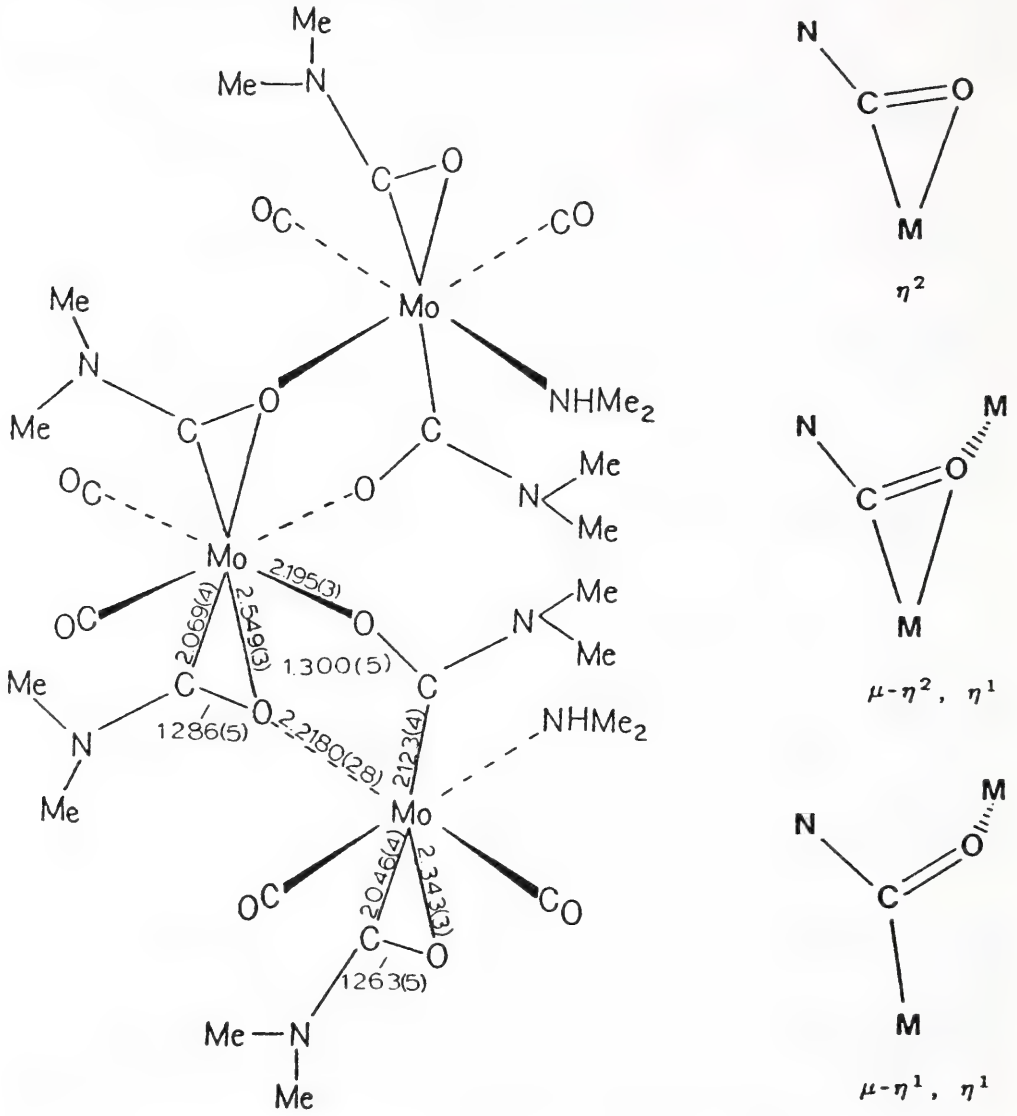

FIGURE 2. Schematic drawing of $Mo_3[C(O)NMe_2]_6(CO)_6(HNMe_2)_2$ with pertinent bond distances for the carbamoyl ligands and drawings of the three types of carbamoyl ligand bonding modes.

In the space group A2/a, the molecule has crystallographically imposed C_2 symmetry. The C_2 axis bisects the *cis*-$Mo(CO)_2$ portion of the central molybdenum atom, Mo(1), and relates the two terminal molybdenum atoms, Mo(2) and Mo(2)′, and their attendant ligands. Each molybdenum atom may be viewed as Mo(2+) if the carbamoyl ligands are counted as uninegative with the carbon-to-metal bond bearing the formal charge. Note that the terminal molybdenum atoms, Mo(2) and Mo(2)′, are bonded to three carbamoyl ligands while the central molybdenum atom, Mo(1), is bonded to four, but each molybdenum atom only makes two Mo-C bonds to carbamoyl ligands. If the η^2-(CO)-carbamoyl ligands are counted as four electron donors and all other carbon-, nitrogen-, and oxygen-to-molybdenum bonds are taken as dative two electron bonds, Mo(2) and Mo(2)′ attain 18 valence electrons while Mo(1) formally has 20 valence elec-

trons. However, the atoms in the two carbamoyl ligands attached to Mo(1) lie in a plane, which together with the relatively long Mo(1)-O distance imply delocalized bonding. If the η^2-(CO)-carbamoyl ligands are presumed to occupy one coordination site, then the local geometry about each molybdenum atom corresponds to a distorted octahedron. The carbamoyl ligand, as seen in this one molecule, can act as an η^2-4 electron donor, a bridging, η^1-C, η^1-O, 4-electron donor, and a bridging, η^2-(CO), η^1-O, formal 6-electron donor as shown in Figure 1. The η^2-(CO) bonding mode has been seen before on a mononuclear actinide center.(5) However, the μ-η^2-(CO),η^1-O and μ-η^1-C,η^1-O are unfamilar bonding modes.

For comparison, the bond distances for the three types of carbamoyl are given in Figure 2. The Mo-C distances of the η^2-ligated carbamoyl ligands are notably shorter than the Mo-C distance of the μ-η^1,η^1-ligand. The C-O distances follow the same relative order as the Mo-C distances. It is interesting to note that the two η^2-carbamoyl ligands are orientated such that their respective (C_2NCO)Mo units are essentially planar. These planes roughly bisect each of the cis-$Mo(CO)_2$ angles.

Isocyanide Reations [C≡NR (R = Me, Et, Pr^i, Bzl)]

The intense purple color of hydrocarbon solutions containing $Mo(NMe_2)_4$ changes instantaneously to brown upon exposure to isocyanides, C≡ (where R = Me, Et, Pr^i, Bzl). Observations by ^{1}H and ^{13}C NMR spectroscopy show the major diamagnetic products formed are 1,1,3,3-tetramethyl-2-alkylguanidines (alkyl- = Me, Et, Pr^i, Bzl), analogous to tetramethylurea, the product formed in the reaction between $Mo(NMe_2)_4$ and C≡O. The product is consistent with a reaction pathway involving (i) C≡NR insertion to give a Mo[Me_2NCNR] moiety and (ii) reductive elimination together with C-N bond formation: "Mo(IV)[Me_2NCNR](NMe_2)" → Mo (II) + $(Me_2N)_2C$=NR. Unreacted $Mo(NMe_2)_4$ in the reaction mixture decomposes to form $HNMe_2$ and other unidentified compounds.

To identify the formation of 1,1,3,3-tetramethyl-2-alkylguanidine (alkyl = Me, Et, Pr^i, and Bzl), 1,1,2,3,3-pentamethylguanidine was synthesized independently, and was added to an NMR tube containing the reaction mixture of $Mo(NMe_2)_4$ and C≡NMe. The resonances in the reaction mixture assigned to 1,1,2,3,3-pentamethylguanidine increased in intensity relative to the solvent protio impurity. The methyl groups of 1,1,3,3-tetramethyl-2-alkylguanidine species have a characteristic pair of singlets (*ca.* δ 2.48 ppm) in the ^{1}H NMR spectrum which are derived from the diastereotopic methyl groups.

tert-Butyl Isocyanide Reactions

In hydrocarbon solutions $Mo(NMe_2)_4$ reacts rapidly with C≡NBu^t (*ca.* 4 equiv) at ambient temperatures producing $Mo_2(\mu\text{-}NMe_2)_2(CNBu^t)_8$ as a red-brown crystalline solid in an isolated 50% yield. When the reaction is carried out in a deuterated solvent, the filtrate shows resonances in the ^{1}H and ^{13}C NMR spectra assignable to 1,1,3,3-tetramethyl-2-*tert*-butylguanidine. The proposed balanced chemical equation is:

$$2Mo(NMe_2)_4 + 11C{\equiv}NBu^t \text{----}> Mo_2(\mu\text{-}NMe_2)_2(CNBu^t)_8 + 3Me_2NC(=NBu^t)NMe_2$$

Upon first examination, the infrared spectrum (mineral oil mull or KBr pellet) does not appear clean; bands assignable to C-N multiple bonds appear at 2045(m brd), 1920(m sh brd), and 1795(vs brd) cm^{-1}. The ^{1}H NMR integral intensities showed the presence of one amido ligand to four icocyanide ligands. The isocyanide ligands are in two environments and exchange positions (T_c = 34.8°, $\triangle G\ddagger$ = 14.9 ± 0.3 kcal/mol) consistent with axial-equatorial ligand exchange.(8) The NMR data are consistent with an edge-shared bioctahedral d^5-d^5 dimer with briding amido ligands and four terminal isocyanide ligands on each molybdenum atom. The molecular configuration was uncertain due to

the broad bands in the infrared spectrum. Therefore, a crystal structure determination was undertaken.

The molecular structure of $Mo_2(\mu\text{-}NMe_2)_2(CNBu^t)_8$ is shown in Figure 3. The structure contains a crystallographically imposed C_2 axis through the bridging nitrogen atoms.

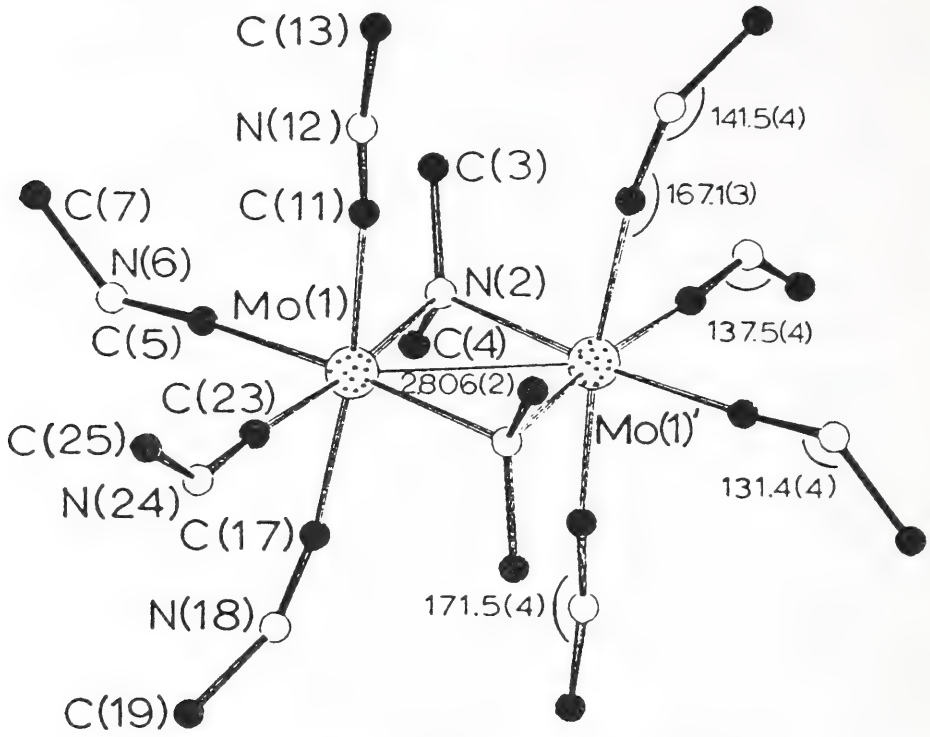

FIGURE 3. Ball-and-stick drawing of $Mo_2(\mu\text{-}NMe_2)_2(CNBu^t)_8$. The *tert*-butyl metyl carbon atoms and all hydrogen atoms are not shown. Pertinent bond distances (Å) and angles (°) are: Mo(l)-N(2) 2.212(3), Mo(l)-N(2)' 2.230(3), Mo(1)-C(5) 1.996(4), Mo(1)-C(23) 2.017(4), Mo(1)-C(17) 2.065(4), Mo(1)-C(11) 2.131(4), C(5)-N(6) 1.213(5), C(23)-N(24) 1.192(5), C(17)-N(18) 1.188(5), C(11)-N(12) 1.166(5), Mo(1)-N(2)-Mo(1)' 78.35(11), N(2)-Mo-N(2)' 101.65(11).

Molybdenum has been reduced from Mo(IV) to Mo(I), presumably by reductive C-N bond formation of $Me_2NC(=NBu^t)NMe_2$. The two molybdenum atoms are in pseudo octahedral environments sharing an edge with a Mo-Mo distance of 2.806 Å corresponding to a Mo-Mo single bond.(3) For comparison we note that related compounds $Mo_2(\mu\text{-}X)_2(CO)_8$, where X = SBu^t (6) and I,(1) have Mo-Mo distances of 2.984 Å and 3.164 Å, respectively. There are four types of isocvanide ligands in the solid state structure accounting for the broad bands in the infrared spectrum.

It is interesting to note that the analogous $Mo_2(\mu\text{-}SBu^t)_2(CNBu^t)_8$ species can be synthesized from $Mo(SBu^t)_4$ and $CNBu^t$, although no crystal structure determination has been undertaken.(6)

Xylyl Isocyanide Reactions

Hydrocarbon solutions of $Mo(NMe_2)_4$ react rapidly with C≡Nxylyl (4 equiv.) at ambient temperatures to give $Mo(\eta^2\text{-}C(NMe_2)Nxylyl)_4$, isolable as pure red-brown crystals in *ca.* 60% yield on cooling the reaction solution to -15 °C.

$$Mo(NMe_2)_4 + 4CNxylyl \text{ -----> } Mo(\eta^2\text{-}C(NMe_2)Nxylyl)_4$$

Crystallization from toluene yields a 1:1 solvate. The infrared spectrum of the crystalline

product showed no bands assignable to $\nu(C\equiv N)$ in the region 2200-1900 cm^{-1}, indicative of C-N or C-C double bond stretches. The ^1H NMR spectrum revealed eight signals of equal integral intensity assignable to methyl groups (NMe_2 and $ArMe_2$), while the ^{13}C NMR spectrum revealed two resonances of equal intensity at δ 213 and 194 ppm assignable to NCN carbons, four amido methyl carbons (*D* 44.8, 43.6, 38.3, and 37.9 ppm), and four aryl-methyl carbons (δ 20.5, 19.9, 19.1, and 18.1 ppm), all of roughly equal intensity.

The molecular structure of Mo(η^2-C(NMe_2)Nxylyl)$_4$ is shown in Figure 4. The central Mo(η^2-C(NC_2)NC)$_4$ skeleton looking down the virtual C_2 axis, which bisects the C(18)-Mo-C(44) angle, is shown in Figure 5. There are two types of amidino ligands. One type haws shorter Mo-C and Mo-N distances relative to the other, but each is bonded to the metal atom in a η^2 manner. The amidino ligands having the shorter Mo-C and Mo-N distances have longer η^2-(C-N) distances than those with long Mo-C and Mo-N distances. The amido nitrogen to isocyanide carbon (Me_2N-CNxylyl) distances are all essentially the same, which together with the planarity of the C_2NCN moieties are indicative of extensive π-delocalization with the ligand.

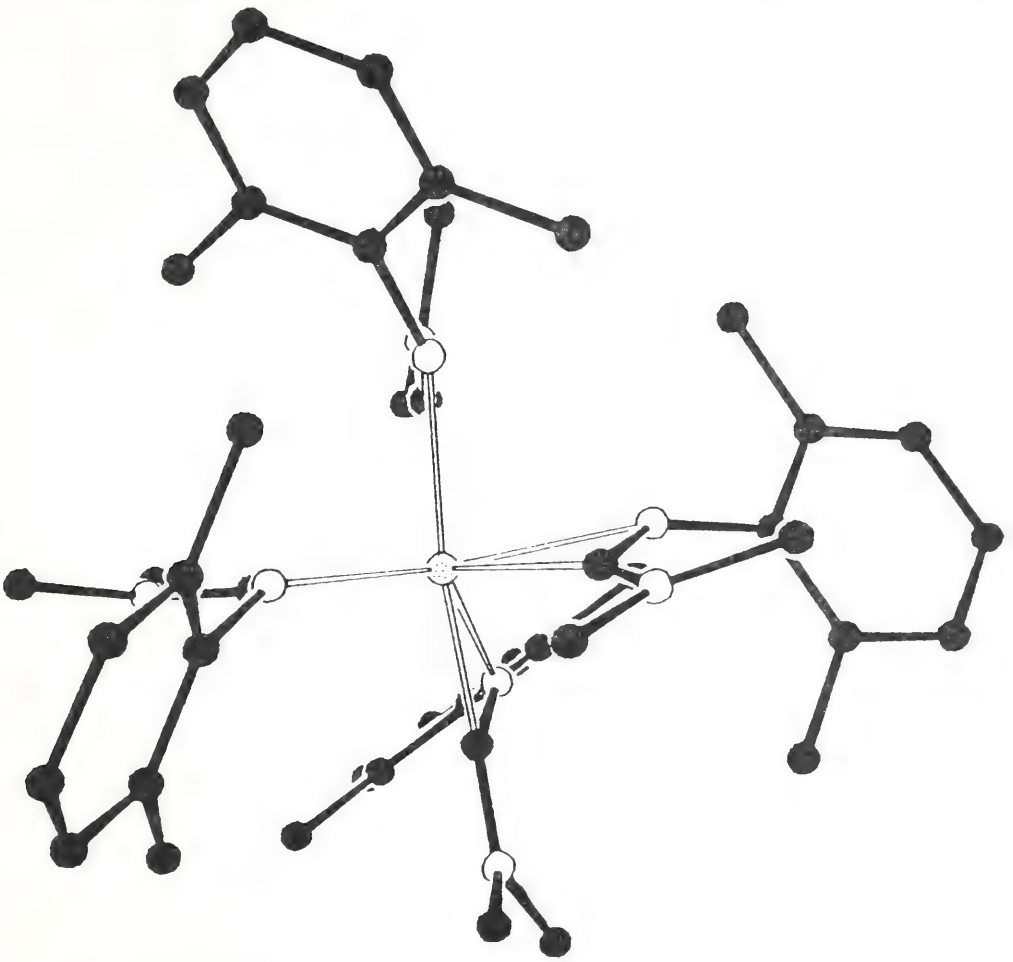

FIGURE 4. Ball-and-stick dragram of Mo(η^2-C(NMe_2)Nxylyl)$_4$ molecule showing the alignment of the four η^2-CN ligands. Viewed down the *z* axis, NCN units are aligned in a pairwise manner along *xz* and *yz* planes.

Mo(η^2-C(NMe_2)Nxylyl)$_4$ provides the first example of 1 a formal insertion of an isocyanide ligand into a M-N bond of a transition-metal amide, 2 a homoleptic

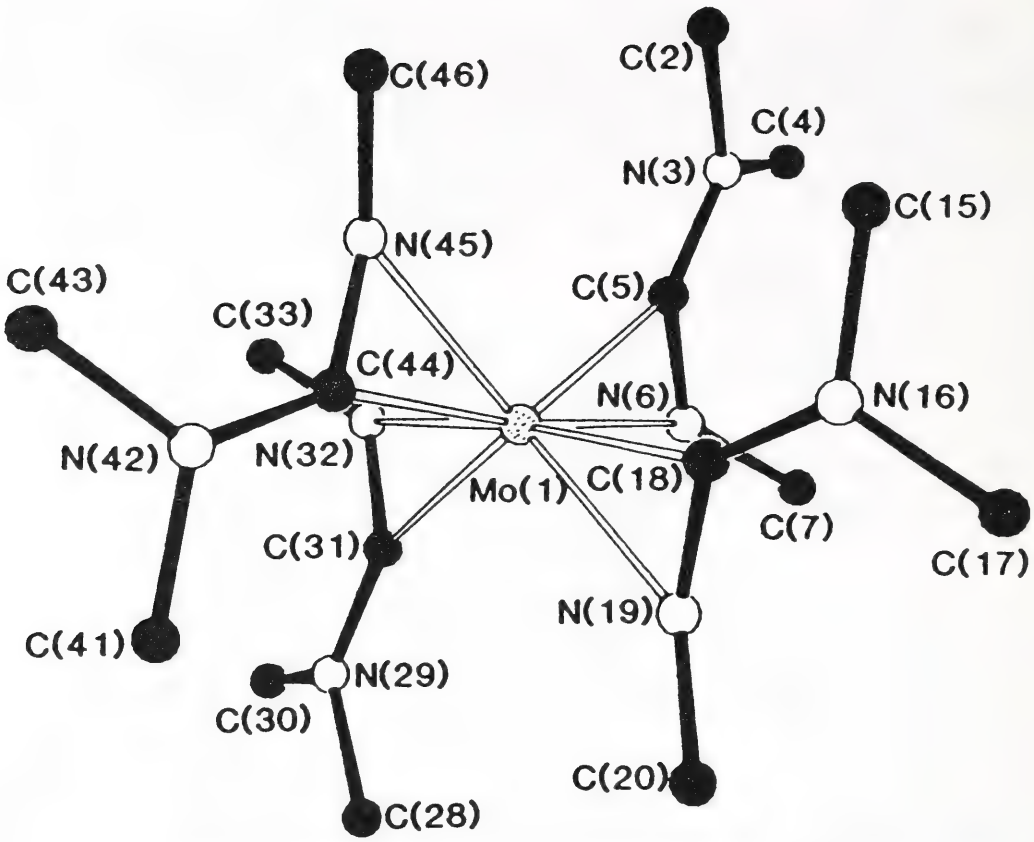

FIGURE 5. View of the central Mo(η^2-C(NC_2)NC) moiety showing the atom number scheme. Pertinent distances (Å): for short Mo-C and Mo-N, Mo-C(18) 2.032(3), Mo-N(19) 2.205(2), Mo-C(44) 2.024(3), Mo-N(45) 2.181(2), η^2-(C-N) 1.336(7) (av); for long Mo-C and Mo-N, Mo-C(5) 2.111(3), Mo-N(6) 2.278(2), Mo-C(31) 2.116(3), Mo-N(32) 2.294(2), η^2-(C-N) 1.297(3) (av) and Me_2N-CNxylyl 1.335(6) (av).

metalloamidine complex, and 3 structural characterization of the η^2-C(NC_2)NR′ ligand. It also provides an example of a C_2 isomer of a distorted dodecahedral Mo(η^2-CN)$_4$ unit which is one of six possible *mmmm* isomers.(9)

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A Simple Flow Coulometric Titrimeter for the Determination of Azo-groups

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Introduction

Azo-dyes and their precursors have always presented a considerable challenge to the analytical chemist especially, as they do so frequently, when they occur in a mixture. The choice of analytical methods becomes one of some complexity because of the fact that many of the analytically useful chemical properties of the precursors are mimicked in the dye, particularly when substituent groups are present.

Historically, this has been the case, and so many methods have been devised to determine dye concentration. For azo-dyes, the property of the precursors not imitated by the dye molecule is the azo-group which, although somewhat unreactive, does possess a chemistry and properties of its own. Use of these properties as an approach to analysis has to be judicious, however, because many of the reagents which act on the azo-group also act on other groups, e.g. titanous chloride which reacts both with azo-groups and nitro-groups. Resorting to microanalysis to determine dye purity has problems of its own: nitrogen or carbon determinations, for example, as means to determine purity would give the same result (very nearly!) for the dye as with a mixture of the two precursor amines, and azo-groups generally give a slightly low nitrogen.

Many approaches to the determination of azo-dyes/groups have been made, one such approach being titrimetry. Knecht and co-workers used successfully a number of very powerful redox reagents: chromium(II) and titanium(III) (5-7). Whilst these reagents were efficient for the purpose, they suffered a number of major drawbacks, not the least of these being their instability towards atmospheric oxygen. In these cases a special device was employed in order to prevent contact between the reagent and oxygen. This is rather a cumbersome device and has to be kept with an atmosphere of hydrogen, thus making it dangerous as well. Even with these precautions the reagents required frequent standardization.

Certain other reductions have been tried, not without some success. For example, Raney nickel has been used as a reduction catalyst by Whitmore and Revukas (11). They investigated its use with a number of dyes and were able to perform complete reductions of the azo-groups to amines. This method, as with that of Knecht (5-7), also worked with substituent nitro- and nitrosyl-groups, which led to some complications. The latter were overcome partially by Early and Ma (2) who used titanium(III) but in hydrochloric acid medium to achieve a degree of reduction selectivity.

Several instrumental methods for azo-dye determination have been developed over the years. Potentiometry is one such technique: several azo-dyes have been titrated in glycerol as a solvent using Mohr's salt as the titrant (8). Flow injection analysis, a more contemporary method, which works by injection of a small quantity of azo-dye into a flowing stream of chemically generated reductant, has produced quite good results (10). Polarography was thought to be a potentially good method because of its ability to supply a continuously variable reducing voltage. It might have produced some interesting hydrazo-compounds, but generally the azo-groups underwent four-electron reductions. Cabral and Turner (1) made a fairly extensive investigation of this method but it has received little use.

Another extremely useful method for azo-dye determination is spectrophotometry, and it has been used in industry in one form or another for at least half a century (9). However, a major problem has always been one of finding a way to purify the dye (4) sufficiently for quantitative use. This difficulty has been overcome at a practical level by following the same industrial preparation process for different batches, and then accepting solutions which have the same absorbances under defined conditions (3).

Experimental

a) Chemicals: All chemicals used were of analytical grade, and all solutions were prepared using distilled water as the solvent. The mercury used for cathode material was distilled twice under reduced pressure and stored in a wide-necked glass bottle with a screw cap. The zinc anode was cast from 99.9% pure zinc metal and was made in the form of a bar approximately 20cm long by 1cm diameter. Acid Red 88 was synthesized specially and was at least 99.9% pure. The potassium dichromate used for titration of the titanium(III) was a primary standard material.

b) Apparatus: The apparatus design was that of a very simple flow system with provision for photometric measurement, a coulometric current supply, and sample introduction from a swan's neck buret (Figure 1). The cell was constructed from a plastic con-

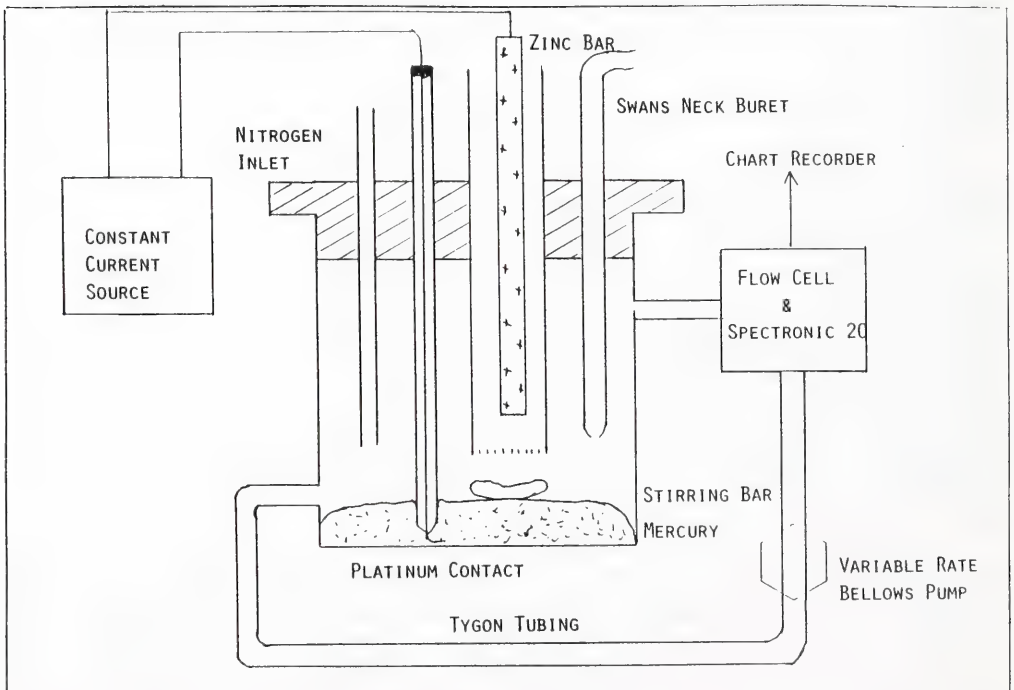
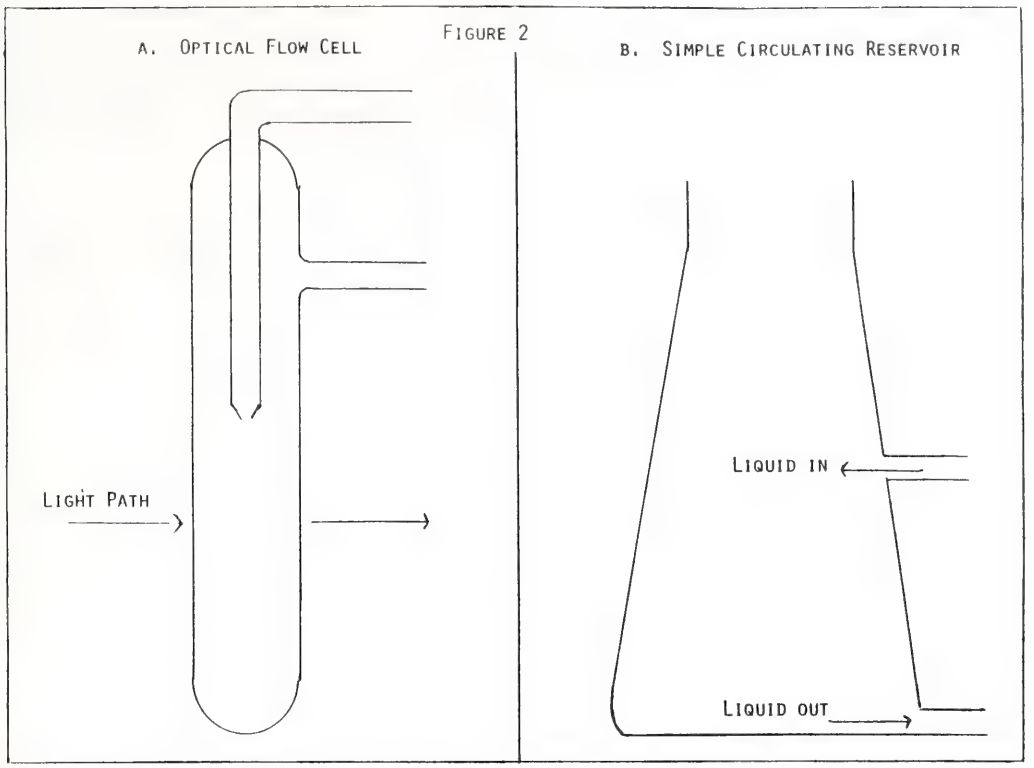


FIGURE 1 DIAGRAM OF FLOW SYSTEM

tainer fitted with a stopper, the latter being bored to accept a fritted glass tube for the zinc anode, the glass insulating tube of the cathode conductor, a glass sparging tube for the introduction of nitrogen gas and a swan's neck buret. The cell was bored to accept two tubes, one at the top of the container and one at the bottom, for entry and exit, respectively, of the circulating solution. The solution in this system was pushed through the circuit by a small bellows pump (Markson Scientific) with a custom built photometric cell in series (Kontes) (Figure 2). The electrodes were connected to a constant current source (Beckman Instruments) which could be switched on as desired. A chart recorder

FIGURE 2



was attached to the Spectronic 20 (Bausch and Lomb) for the purpose of continuously recording the solution absorbance. The cathode connector was a glass tube with a short length of platinum wire sealed into one end. This end was well submerged in the mercury pool cathode and the other end was connected to the coulometer using the conventional mercury and copper wire.

c) Testing the Apparatus: The apparatus was tested in two ways: flow rate and electrolysis efficiency. In the first case, ordinary distilled water was allowed to flow through the system and a small quantity of dye solution was introduced. The time taken for the dye to mix completely, i.e. achieve a constant absorbance value, was noted for different flow rates, and ones of 25ml/min. or faster were found to promote the best mixing conditions. Subsequently, appropriate mixing/flow conditions could be selected before each experiment.

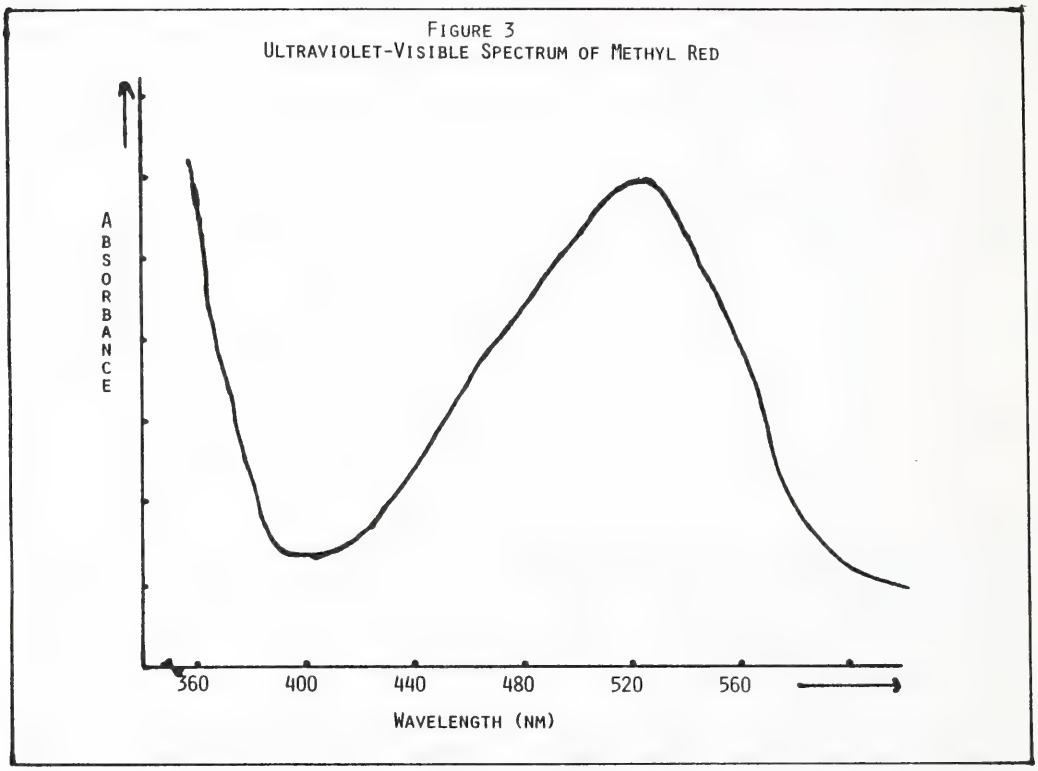
With regard to electrolysis efficiency, the cell was set up with a solution of titanium(IV) chloride in hydrochloric acid ($\doteq$4M) circulating at an appropriate rate, and it was then electrolyzed for various periods of time at constant current. Throughout the electrolysis nitrogen gas was flushed through the system to provide an inert blanket of protection for the titanium(III) generated. The current was measured by putting a standard resistance across the coulometer leads and measuring the potential difference between its ends with a reliable high impedence volt meter. The most frequently used current was 99.87mA. After a period of electrolysis, a sample of the solution was removed from the cell and titrated with standard potassium dichromate solution to determine its titanium(III) concentration. The results of these tests are recorded in Table 1.

d) Performing an Experiment: In order to titrate an azo-dye, a small quantity of its solution was introduced into the cell from the swan's neck buret. Nitrogen gas was blown through the cell for a few minutes prior to commencing the experiment, and then during it as well. The spectrophotometer was set at a suitable wavelength decided upon by a previous experiment (Figure 3), and electrolysis was started. The resulting curve obtained from

TABLE 1. Efficiency of Coulometric Titration*

Time (secs)	Microeqs. Ti	Calcd. Microeqs. Ti	Efficiency (%)
210.6	217.55	217.99	99.8
268.3	275.77	277.72	99.3
179.4	184.58	185.70	99.4
239.1	245.26	247.49	99.1

*Grand average over fifteen titrations = (99.6 + 0.3)%. This was close enough to assume 100% electrolysis efficiency throughout the experiments.



the chart recording is shown in Figure 4 along with its interpretation. Both very pure Acid Red 88 (Figure 5a) and highly impure Methyl Red (Figure 5b) were investigated in this way.

Results and Discussion

Figure 4 shows the results of a flow experiment in which the points at which the current was turned on and off are shown. As the azo-group is reduced, the absorption due to the dye decreases until a constant value is attained. In practice the curves obtained were virtually identical to this curve. Time of electrolysis was read from both this graph and the "digital" output of the coulometer, this being the major analytical parameter; the other was the applied current, which was measured at 99.87mA for most of the experiments.

Table 2 shows the results of several experiments with extremely pure Acid Red 88 in which several different but known quantities of the dye were reduced using the electrogenerated titanium(III). The series of reactions conjectured to be involved was:

(i) $Ti^{4+} + e \rightleftharpoons Ti^{3+}$ (generated at the mercury cathode)

(ii) $Zn \rightleftharpoons Zn^{2+} + 2e$ (sacrificial anode)

(iii) $4Ti^{3+} + \text{-N=N-} + 4H^{+} \rightleftharpoons 4Ti^{4+} + \text{-}NH_2 + H_2N\text{-}$

or (iv) $2Ti^{3+} + \text{-N=N-} + 2H^{+} \rightleftharpoons 2Ti^{4+} + \text{-NH-NH-}$

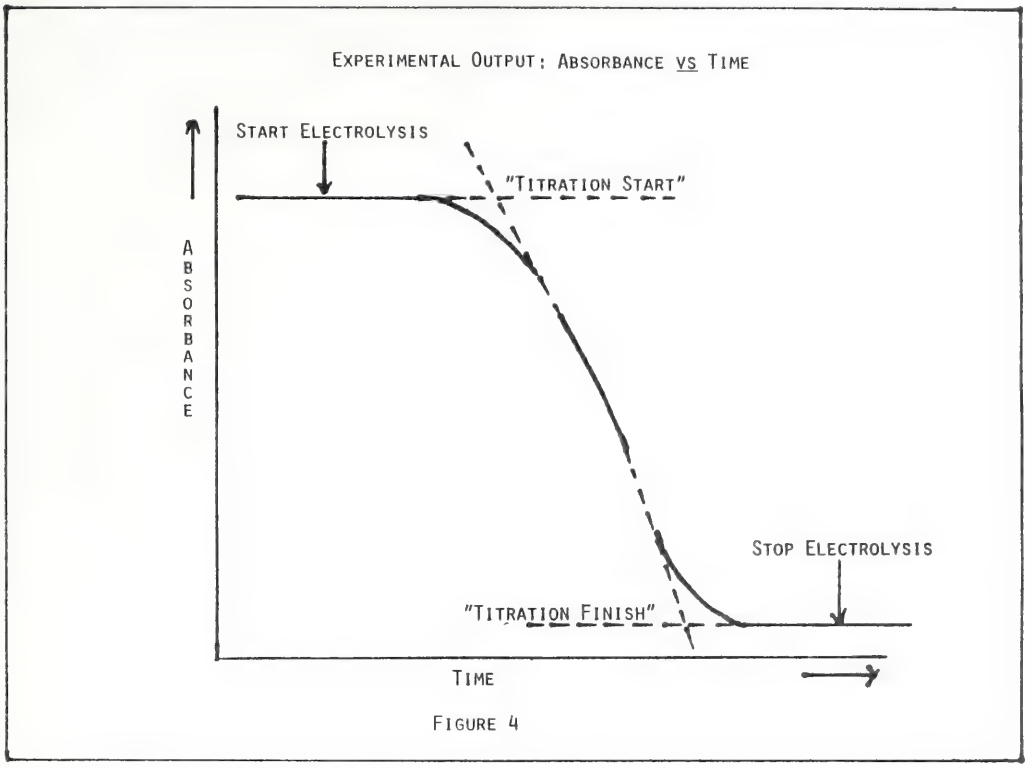


FIGURE 4

TABLE 2. Reduction of Acid Red 88*

mls of Dye Soln.	Time (secs)	Microeqs. Dye	Number of Electrons
10.00	148.3	153.50	4.0
12.50	185.7	192.22	4.0
15.00	221.6	229.38	4.0
17.50	260.6	269.75	4.0
20.00	294.1	304.42	4.0

*1.5352g/l of dye; current = 99.87mA; these are examples of the results of many experiments.

Using Faraday's Laws and knowing the quantity of dye present, as well as the applied current, allowed the number of electrons involved in the reduction to be calculated. Examples of the values obtained are shown in Table 2 and illustrate that the reaction of Acid Red 88 is one involving four rather than two electrons. Thus reaction (iii) rather than reaction (iv) is the case.

Methyl Red (Figure 5b) was chosen as another dye for investigation. Its structure shows similarities to that of Acid Red 88 (Figure 5a), and thus it might be expected to undergo a reduction similar to that of Acid Red 88. During the experiments with Methyl Red it was assumed that it would undergo a four electron reduction, and latterly this was found to be true based upon a little spectroscopic evidence obtained at a later stage. Basically, several spectroscopic experiments were done to determine the purity of the impure Methyl Red, and they were found to confirm the electrochemical ones, in short indicating that the assumption of a four electron reduction was accurate. Table 3 shows the results of several experiments with Methyl Red. Its purity is quite low, but this is to be expected with some dyes through photolysis, salting-out procedures and general age.

In conclusion, a simple method and apparatus have been described for the determination of azo-groups, and hence certain azo-dyes. Unfortunately, the method cannot

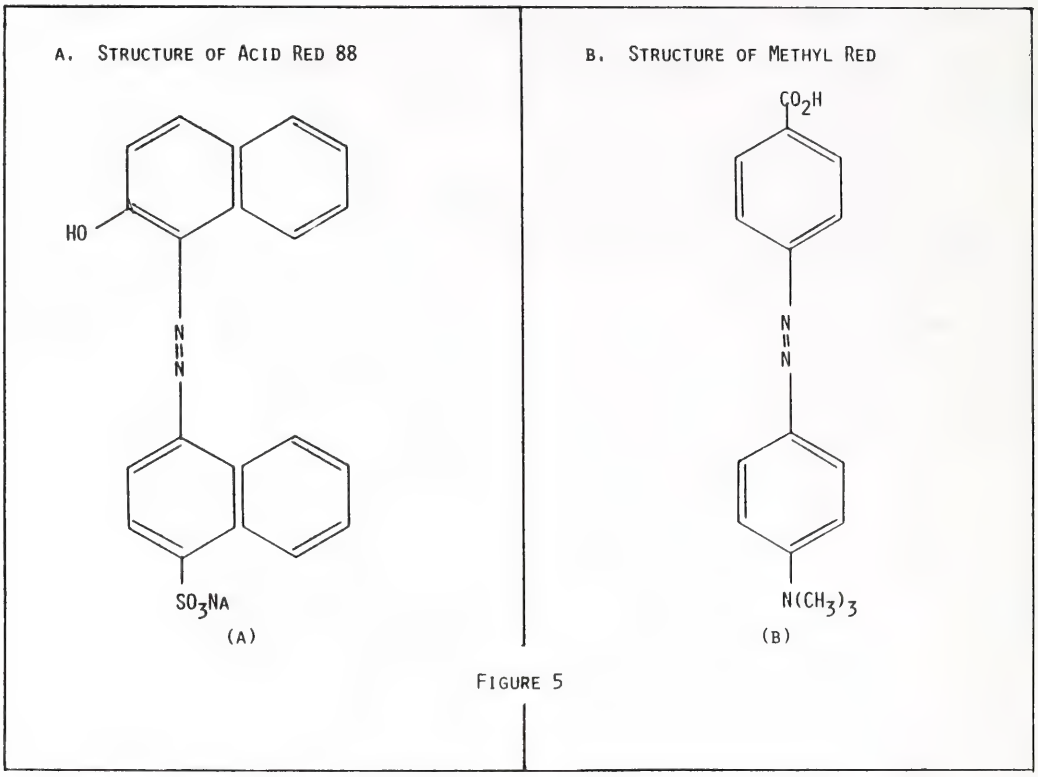


Figure 5

Table 3. Reduction of Methyl Red*

mls of Dye	Time (secs)	Microeqs. Dye	Purity (%)
10.00	131.2	135.81	33.05
10.00	129.7	134.24	32.67
20.00	263.2	272.38	33.15
20.00	262.4	271.62	33.05

*2.9378g/l of dye; assuming that it is a four electron reduction; these are examples of the results of many experiments.

be used with all azo-dyes owing to the possible presence of other reducible groups, for example nitro-groups. The unknown presence of these groups would be a major source of error, and even if their presence were known it would still present a problem unless a way to account for them were to be developed.

Acknowledgments

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Chromatography/SIMS: Applications to Peptides and Bile Acids

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Introduction

Biological molecules are often analyzed by fast atom bombardment (FAB) and secondary ion mass spectrometry (SIMS), both of which are "soft" ionization techniques that produce abundant ions containing molecular weight information. Fragment ions are of low abundance relative to the molecular ions, and, in the case of peptides, such ions can be interpreted to provide the amino acid sequence. The biological molecules analyzed by FAB and SIMS are incompatible with separation by gas chromatography. Liquid chromatography can be used, but the interface to mass spectrometry is not yet perfected. We have constructed a chromatography/SIMS instrument that directs a beam of primary ions onto a thin-layer chromatogram positioned inside the instrument (3), and have used this instrument for the analysis of mixtures of peptides and bile acids. Secondary ions are sputtered from the intact thin-layer chromatogram with the aid of a phase-transition matrix (2), and are similar in appearance to the mass spectra of the pure samples in glycerol. In addition to the molecular weight and structural information, the x and y spatial profile of the ions in the mass spectrum can be measured, providing an organic ion image of the surface. A complete mass spectrum can also be obtained at each point in the chromatogram. This SIMS detection system offers unique advantages in comparison to conventional methods of TLC analysis and other bioanalytical techniques such as GC/MS. These advantages include: 1) independent access order to sample spots, 2) variable spatial resolution, 3) preservation of chromatogram integrity, 4) variable integration time, and 5) four-dimensional data: x, y in distance, mass-to-charge ratio, and ion intensity or relative abundance.

Experimental

SIMS mass spectra were obtained by dissolving approximately 1 mg sample into 0.3 mL glycerol or a molten mixture of sorbitol and p-toluene sulfonic acid. Spectra were obtained using a modified Extranuclear mass spectrometer equipped with a commercial cesium ion gun (Phrasor Scientific). TLC/SIMS data was obtained by depositing 100 ug sample onto a silica gel (0.2 mm) thin layer chromatogram. The ion source used to obtained the three-dimensional image was a liquid gallium ion gun manufactured by FEI.

Leucine enkephalin and methionine enkephalin were obtained from Sigma Chemical Co. Cholic acid, chenodeoxycholic acid, lithocholic acid and various solvents used were obtained from Aldrich Chemical Co. All were used without further purification.

Results and Discussion

The SIMS mass spectra of the bile acids studied show no protonated molecules; instead loss of H_2O occurs readily. The SIMS spectrum of pure cholic acid, the trihydroxy bile acid, shows as the base peak the $(M + H - 3H_2O)^+$ ion (m/z 355) and another prominent ion at m/z 373 which corresponds to $(M + H - 2H_2O)^+$. Chenodeoxycholic acid, the dihydroxy species, shows one ion at m/z 357 corresponding to $(M + H - 2H_2O)^+$. Lithocholic acid, the monohydroxy species, shows only ions that correspond to the incorporation of sodium ions. Thus, to obtain this spectrum, the glycerol matrix was doped

with NaCl. The base peak is m/z 421, $(M + 2Na - H)^+$. The three-dimensional image of the three bile acids separated on a thin-layer chromatogram was then obtained (Figure 1). By moving the platform in successive steps in x and maintaining a constant y, and

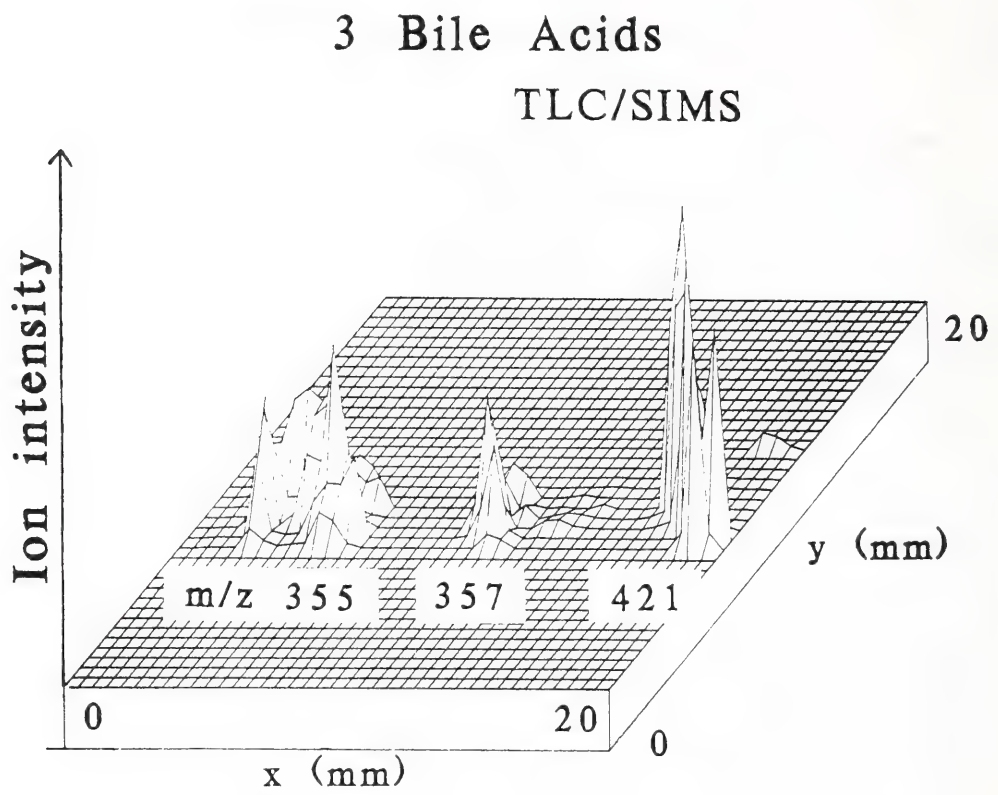

FIGURE 1. Three-dimensional image of the cholic acid (left), chenodeoxycholic acid (center) and lithocholic acid on a silica thin layer chromatogram.

in monitoring the appropriate ion for each acid, the spatially-resolved image was measured. The procedure was repeated changing the y value until an entire image was obtained. The figure shows that the spots are completely resolved, and signal-to-noise ratios are excellent. In addition, the size of each chromatographic spot can be determined by inspection. As mentioned above, data is generated in four dimensions: x, y, m/z, and ion intensity.

FAB and SIMS mass spectra of peptides typically contain the protonated molecule of the peptide and fragment ions that correspond to sequential cleavages at the peptide bonds (1). In this way, the amino acid sequence of a given peptide can be deduced from the masses of the fragment ions. Figure 2 is the SIMS spectrum of the pentapeptide leucine enkephalin taken directly from a TLC plate. The protonated molecule is seen at m/z 556, and the $(M + Na)^+$ is seen at m/z 578. Sodium is an impurity often present in TLC plates. A prominent fragment ion is observed at m/z 278, and the ion labeled 303 and the other, smaller ions seen are either system contaminants or matrix ions. Methionine enkephalin (Tyr-Gly-Gly-Phe-Met), also shows a protonated molecular ion at m/z 574 and a fragment ion at m/z 278, which is the same ion as that in the spectrum of leucine enkephalin; it corresponds to loss of the 2 C-terminal amino acids leaving $(Tyr\text{-}Gly\text{-}Gly + H)^+$. A three-dimensional image of the 2 enkephalins separated on a TLC plate was obtained by monitoring this fragment ion, as shown in Figure 3.

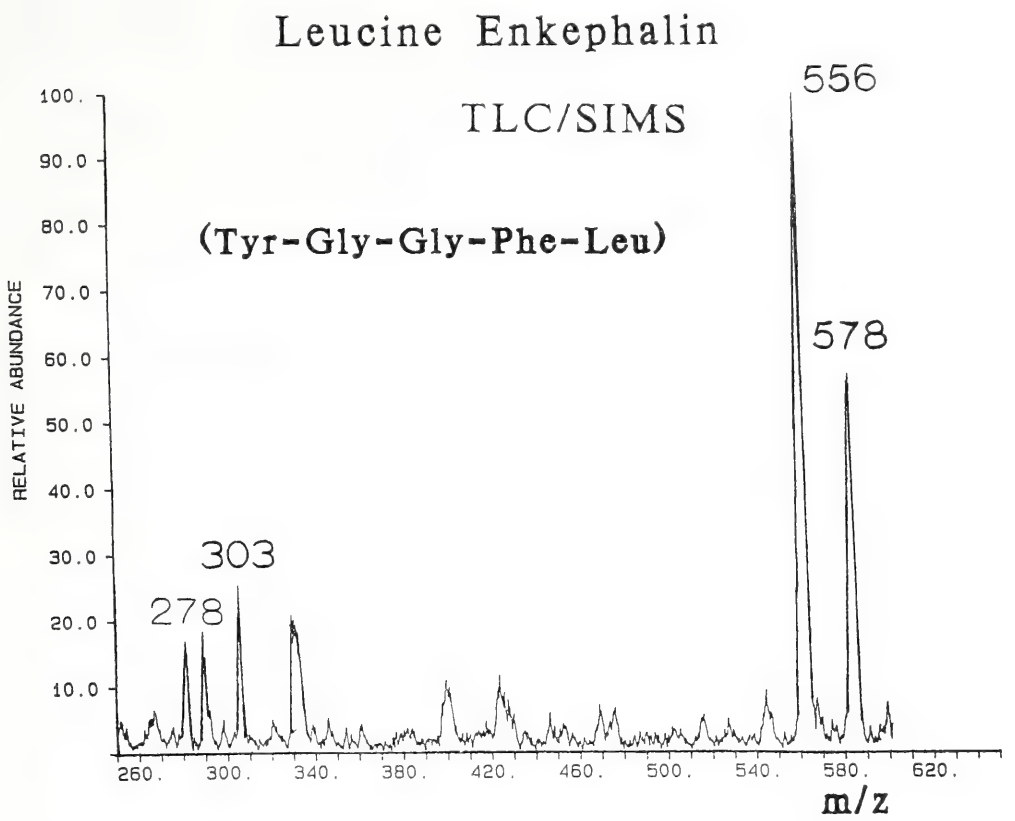

FIGURE 2. TLC/SIMS spectrum of leucine enkephalin.

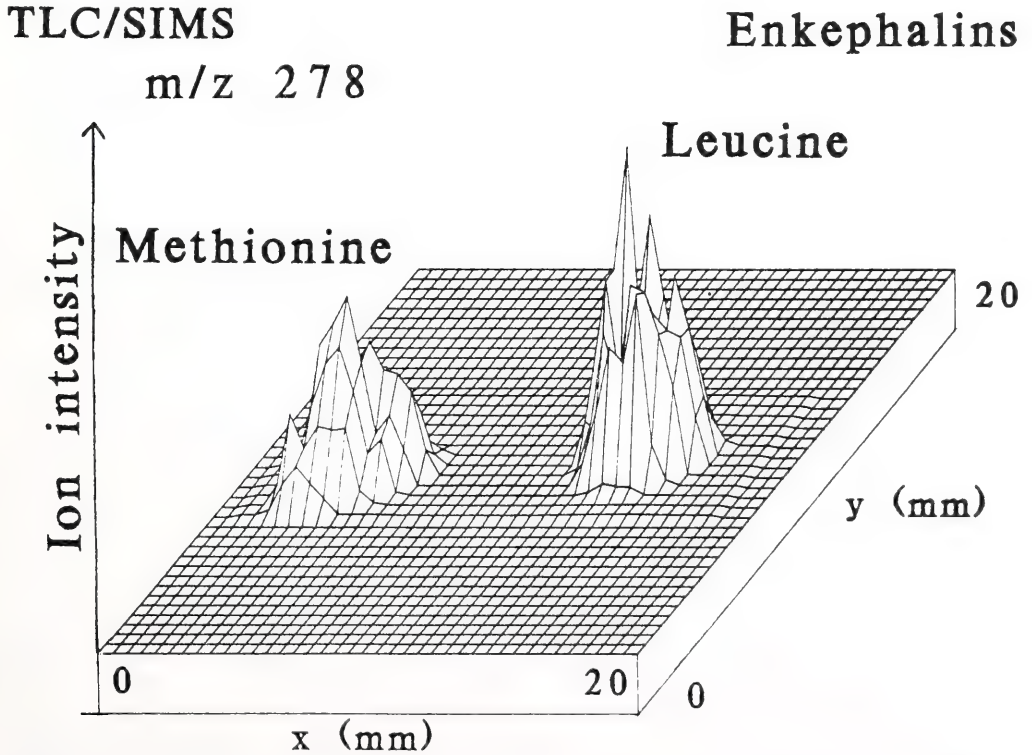

FIGURE 3. Three-dimensional image of leucine and methionine enkephalin on a silica thin layer chromatogram.

This figure illustrates an application of SIMS termed "spatially resolved selected sequence monitoring" of peptides. As shown, each peptide that dissociates to form an ion of specified sequence, selected by mass, will produce a local maximum in ion abundance in the three-dimensional plot. Peptides that are incompletely separated by the chromatography, or even those that exceed the mass range of the mass spectrometer but that still fragment to produce an ion of that specified sequence, can be identified. Thus, enkephalin derivatives would be distinguished from other peptides by monitoring the ion at m/z 278. Experiments continue in the development of this unique application.

Conclusions

Chromatography/SIMS is a viable technique for the analysis of biomolecules. Three-dimensional organic images of the compounds separated by TLC can be generated without substance elution. Chromatogram integrity is maintained, and repetitive analyses can be completed, making feasible important new experiments such as the spatially resolved selected sequence monitoring.

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Evolution of Water Vapor from Complex Solutions of Concentrated Acids

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Introduction

In the commercial production of phosphoric acid from phosphate ore and sulfuric acid, various silico-fluorides are produced (1,3,7). Vapor phase recovery of the volatile silico-flourides presents an opportunity to obtain several compounds of significant commercial value, including high purity silicon, silicon tetrafluoride and hydroflouric acid (4,6,8). At present, the only one of these potential by-products recovered commercially is fluosilicic acid (2). A question central to the development of recovery processes, for other by-products, is the equilibrium of the vapor phase composition above process streams of interest. These have not been previously reported.

A major difficulty in the investigation of these systems is the highly corrosive nature of the liquid and the gas phases. Fourier transform infrared spectroscopy (FTIR) was used to follow the vapor pressure of the key species of interest over a liquid which simulated a commercial process stream. FTIR provides a convenient, non-invasive means of investigating the vapor phase composition.

Among the key species of interest is water vapor. Dühring's Rule, an empirical method to reduce the number of measurements needed to determine the temperature dependence of vapor pressure, was reported over a century ago (5). However, it does not apply to systems with more than one volatile species and there is no evidence that it can be relied upon to describe the system of mixed acids of interest. Accordingly, it was necessary to determine the vapor pressure of water as a function of temperature for the single solution composition targeted for the recovery process and to use these data to test the applicability of Dühring's Rule for our system.

Materials and Methods

A specially designed gas-tight cell was constructed to contain both the liquid and the gas phase. All portions of the cell contacting either phase were constructed of polytetrafluoro ethylene (PTFE). The cell was fitted with an external heater controlled by a Digitech 2770A controller with the control thermocouple along the exterior of the cell. The temperature of the gas phase was measured by a PTFE coated thermocouple placed in the cell head space. The basic cell construction is shown in Figure 1.

The bottom one third of the cell windows was obscured with PTFE tape to prevent the spectrometer beam from encountering the liquid phase in the cell and to protect the windows from contact with the liquid phase. The cell windows used were zinc selenide. Sodium chloride windows can also be used but they must be kept at or above the cell temperature to prevent condensation and resulting erosion of the windows.

The simulated process stream mixture was made from ACS reagent grade phosphoric acid, sulfuric acid, hydrofluoric acid, high purity precipitated silica, and deionized water. To prevent the loss of volatile species during mixing after addition of sulfuric acid, and during storage, the mixture was cooled below O °C whenever not in a closed container.

Each experiment began with the cell at ambient temperature. The cell was purged with ultra-high purity helium while monitoring the water and carbon dioxide peaks until they stabilized. At that point the background spectrum was taken and the cell was charged with 2 ml of the simulated process mixture. FTIR spectra were collected at about 2 minute

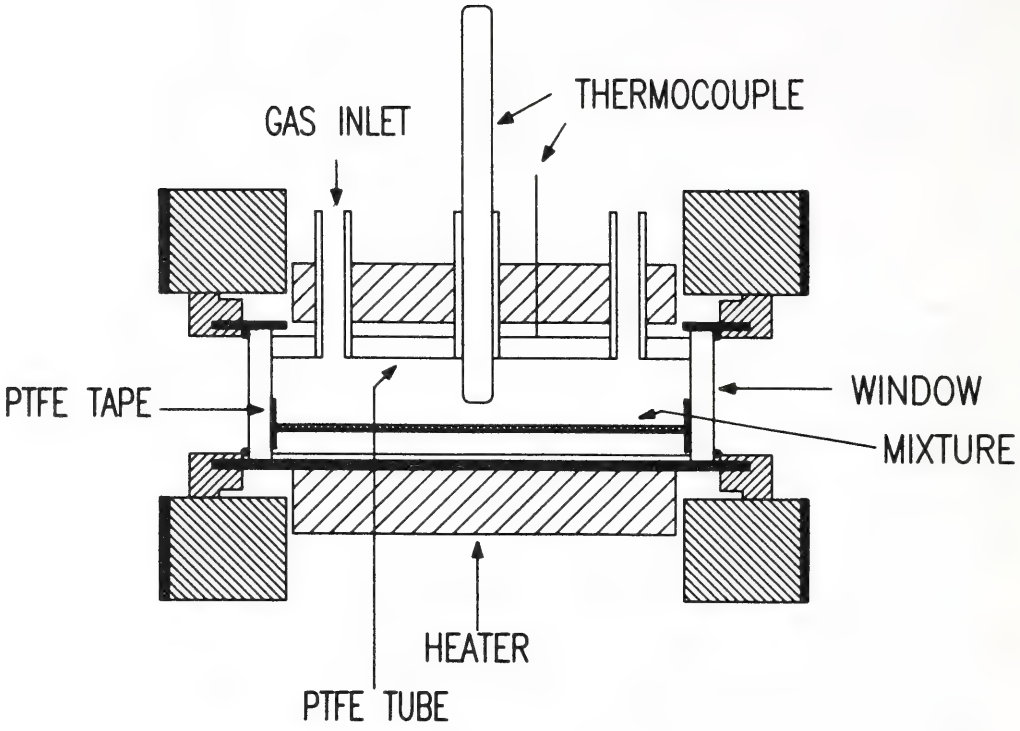

FIGURE 1. Heated FTIR sample cell in cross section. The optical path is in the plane of the drawing from right to left.

intervals while the temperature of the cell was increased to 100 °C at 0.5 °C per minute. The temperature differential between the exterior cell wall and the vapor phase was about 2 °C during the heating cycle.

FTIR spectra were collected using a Nicolet 170SX spectrometer at 4 cm^{-1} resolution. The spectra from sample measurements were ratioed to a spectrum taken through the empty cell, thus effecting an optical null. The integrated area of the spectral region from 1850 to 1240 cm^{-1} was used to measure the water vapor concentration of the vapor phase.

Results

The cell constant for water vapor concentration was determined using deionized water over the temperature range from 32 °C to 100 °C. The constant, 1.17, was determined by plotting the log of the integrated absorbance versus the log of published vapor pressure data. This calibration was checked using a mixture of 75.5% sulfuric acid in water. Figure 2 shows good agreement between measured and published values (9).

The vapor pressure of water above a simulated process stream containing 42% H_2SO_4, 16% H_3PO_4, 1% HF, and 0.5% SiO_2 is shown on a Dühring plot in Figure 3. In this representation of the data, the abcissa is the temperature of the mixture and the ordinate is the temperature at which a reference liquid (water in this instance) has the same vapor pressure as the mixture. If the mixture follows Dühring's Rule, the vapor pressure as a function of temperature may be determined from a plot of two measurements made at different temperatures and the points will fall on a straight line. Figure 3 shows that Dühring's Rule applies with reasonable precision to the water vapor pressure above the mixed acid system. The infrared spectrum of the process stream clearly displays volatile

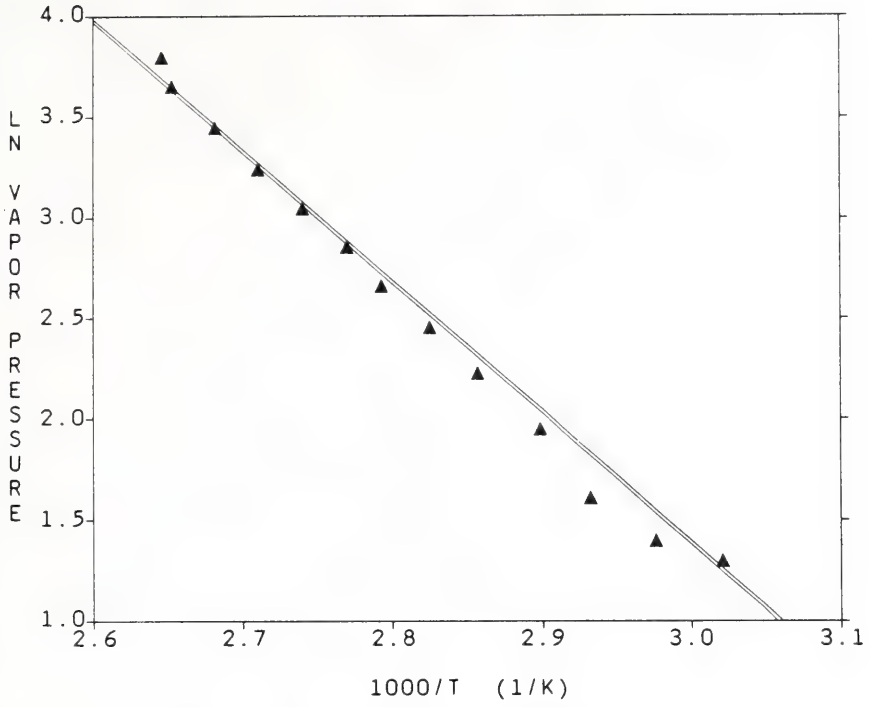

FIGURE 2. Water vapor pressure above a 75.5 percent solution of sulfuric acid: points represent measured values, the line represents published values (9).

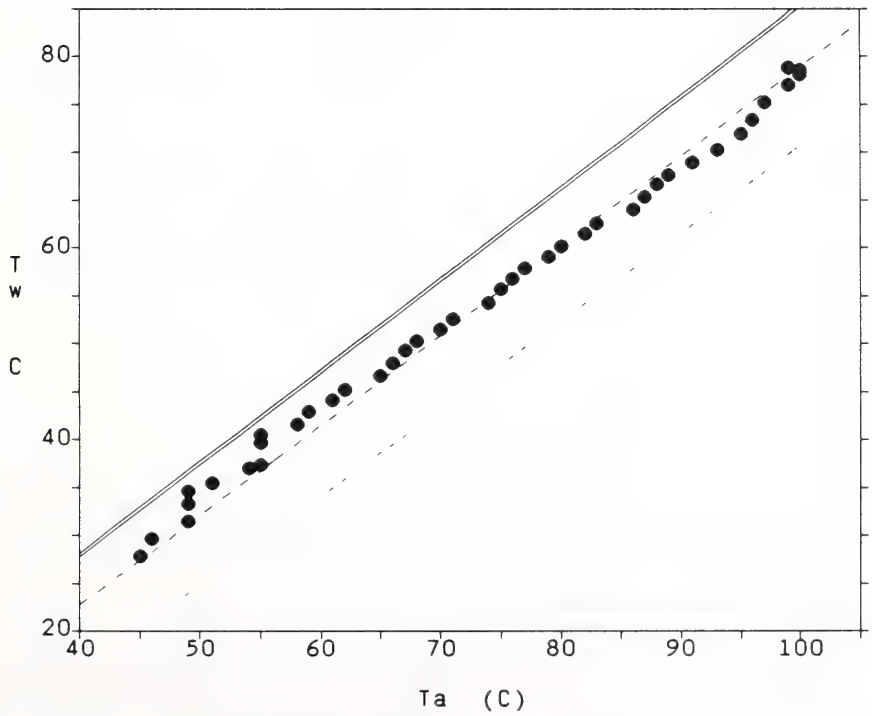

FIGURE 3. Dühring plot of the vapor pressure of water above an aqueous mixture containing 42% sulfuric acid, 16% phosphoric acid, 1% silicon dioxide, and 0.5% hydrogen fluoride: solid line—published values for 42% sulfuric acid, dotted line—published values for 56% sulfuric acid, dashed line—published values for 49% sulfuric acid (9).

species in addition to water. There is a large peak due to SiF_4 and a small peak which has not been identified but is consistent with HF.

For practical purposes, it would be convenient to be able to estimate the vapor pressure of mixtures having compositions similar to the one tested. The lines in Figure 3 represent published data for the vapor pressure of aqueous sulfuric acid solutions. The upper line is for sulfuric acid at the same concentration as the synthetic mixture. The lower line is equal in concentration to the sum of sulfuric and phosphoric acids. An effective sulfuric acid concentration of the mix may be determined by recalculating the sulfuric acid normalized by ignoring the phosphoric acid. The center line is calculated using the effective sulfuric acid concentration. The free energy, enthalpy, and entropy of evaporation are all the same as those of pure water, within experimental error (5%).

Discussion

The heated PTFE cell provides a satisfactory means of measuring the vapor pressure of water over highly corrosive liquids. These data are sufficient to permit systematic design of processes for recovering high-purity silico-fluorides from the production of phosphoric acid. The mixed acid system studied obeys Dühring's rule. Thus, simplifying the determination of water vapor pressure above the condensed phase as a function of temperature. For the mixed acid system, with about 40% sulfuric acid and less than about 20% phosphoric acid, the vapor pressure can be described by the published data for the effective sulfuric acid concentration, normalized by ignoring the phosphoric acid. Energy requirements for water evaporation in the recovery of fluoro-silicates can be calculated from data for pure water. Additional work which would assist development of recovery processes includes; measurement of the vapor pressure of actual process streams, identification of other volatile species, and quantitation of silicon and fluorine containing species.

Acknowledgment

Jerome L. Martin contributed to analysis of experimental results and suggested application of Dühring's Rule.

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Fourier Transform Infrared Studies of Halogenated Quinone Charge-transfer Complexes

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Introduction

Quinone molecules contain a highly conjugated π system which includes both the ring carbon-carbon bonds and the carbon-oxygen double bonds (carbonyl groups). The antibonding orbitals of this delocalized π system give the quinone structure the capacity to function as a Lewis Acid and accept electron density from donor molecules. This transfer of electron density from the donor molecule to the quinone acceptor forms a charge-transfer bond between the two molecules and results in the formation of a charge-transfer complex (1). The increase of the electron density in the quinone antibonding orbitals causes the bond order of the carbon-oxygen double bonds on the quinone molecule to decrease and results in the decrease in the infrared vibrational frequency of the carbonyl bonds. The magnitude of the decrease in both the bond order and the vibrational frequency of the carbonyl bond is dependent on the extent of electron density transferred, the strength of the acceptor, and the strength of the donor, and it reflects the stability of the charge-transfer complex.

The quinone structure is a basic unit of a number of biologically interesting compounds, vitamin K, plastoquinone, and coenzyme Q. The study of the charge-transfer complexes formed between the quinone structure and donor molecules is important to the understanding of the kind of bonding that takes place in a number biologically interesting processes (1, 4, 2, 7, 8, 9). In this study the charge-transfer complexes of tetrahalo-1,4-benzoquinones (*p*-fluoranil, *p*-chloranil, and *p*-bromanil) and a series of donor molecules (polyalkylated aromatic and a special amine super donor, tetrakis(dimethylamino)ethylene) were prepared, and Fourier transform infrared spectroscopy was used to determine the position of the quinone carbonyl vibrational absorption in the complexes. A sandwich structure of the aromatic-quinone charge-transfer complex is pictured in Figure 1. The magnitudes of the shifts to lower wavenumbers of the

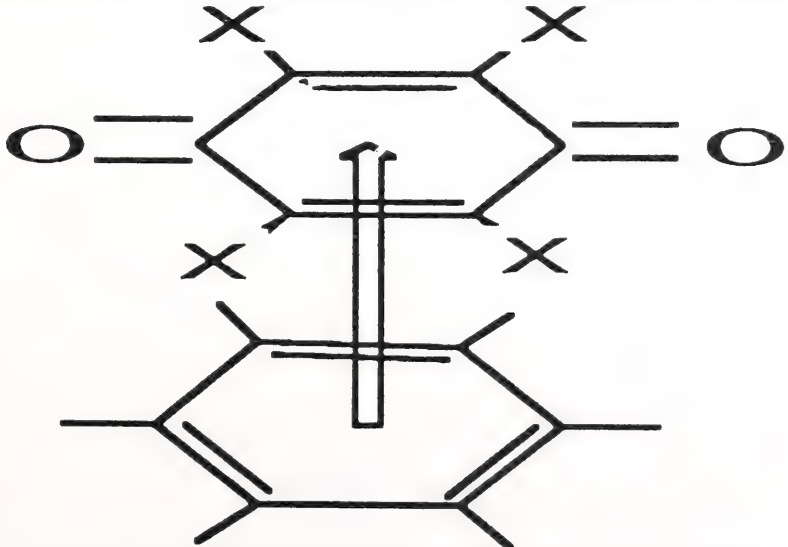

Figure 1. A Tetrahalo-1,4-benzoquinone-aromatic Charge-Transfer Complex.

carbonyl stretching absorptions in the complexes relative to the same stretching absorption in uncomplexed quinones were determined and related to the strength of the donor, the strength of the acceptor, and the stability of the complex.

Experimental

Each of the quinone-aromatic complexes was prepared by dissolving equimolar amounts of the quinone and aromatic compound in dichloromethane and allowing the resulting solution to evaporate. Tetrakis(dimethylamino)ethylene is an oxygen sensitive compound, and therefore its quinone complexes were prepared in an inert atmosphere glove box following the method described in the literature by H. E. Winberg (10). Although tetrakis(dimethylamino)ethylene is air sensitive, its complexes with the halogenated quinones were stable enough in the atmosphere so that no special methods were needed to record their infrared spectrum.

The infrared spectra of the complexes were recorded on a Digilab FTS 40 Fourier Transform Infrared Spectrometer. The spectra of the complexes were collected at 2 cm^{-1} resolution in a KBr matrix. The complex concentrations in KBr were adjusted to be 2% by mass for each complex. Multiple sample preparations were carried out for each complex and the spectra recorded for each of these samples to determine the dependence of the position of the carbonyl absorption on the complex concentration in the KBr matrix. For the experimental variations in concentration of the complexes due to sample preparation, no more than a 0.2 cm^{-1} shift in the carbonyl absorptions was observed due to matrix effects.

Results and Discussion

Fourier transform infrared spectroscopy with its high sensitivity and its ability to determine accurately the positions of vibrational absorption bands is well suited for this type of study of the stability of the charge-transfer complexes. The vibrational absorptions of the quinone carbonyl bonds in these charge-transfer complexes are located in a region from 1700 to 1650 cm^{-1}. Figures 2-4 show the spectra of the carbonyl bonds

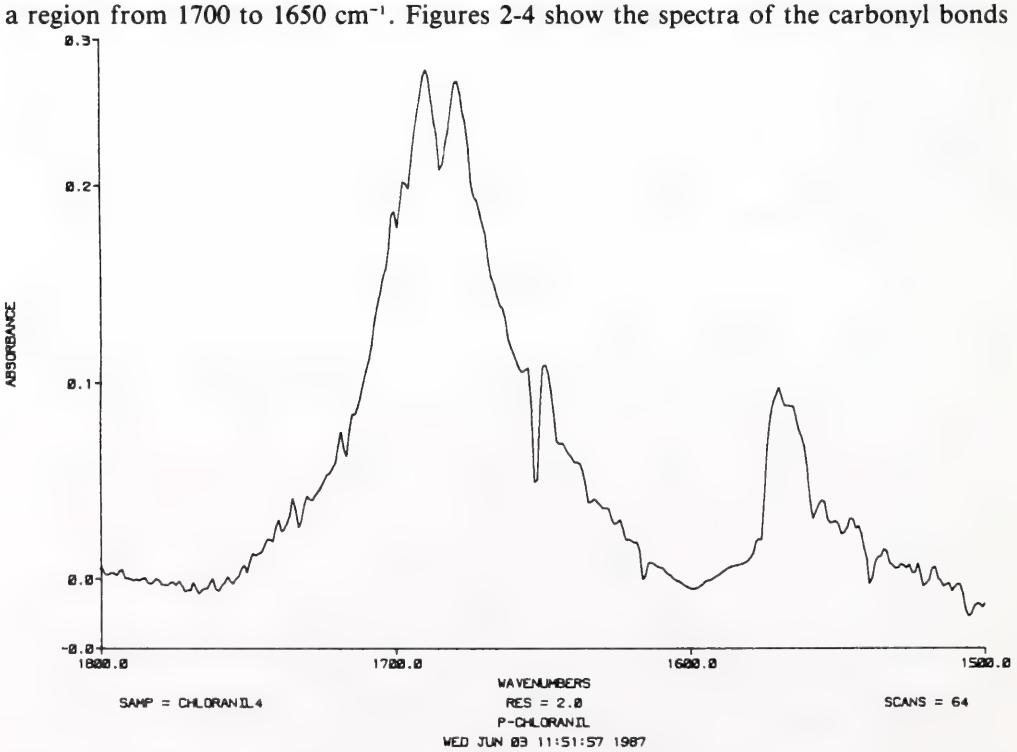

FIGURE 2. Spectra of Chloranil.

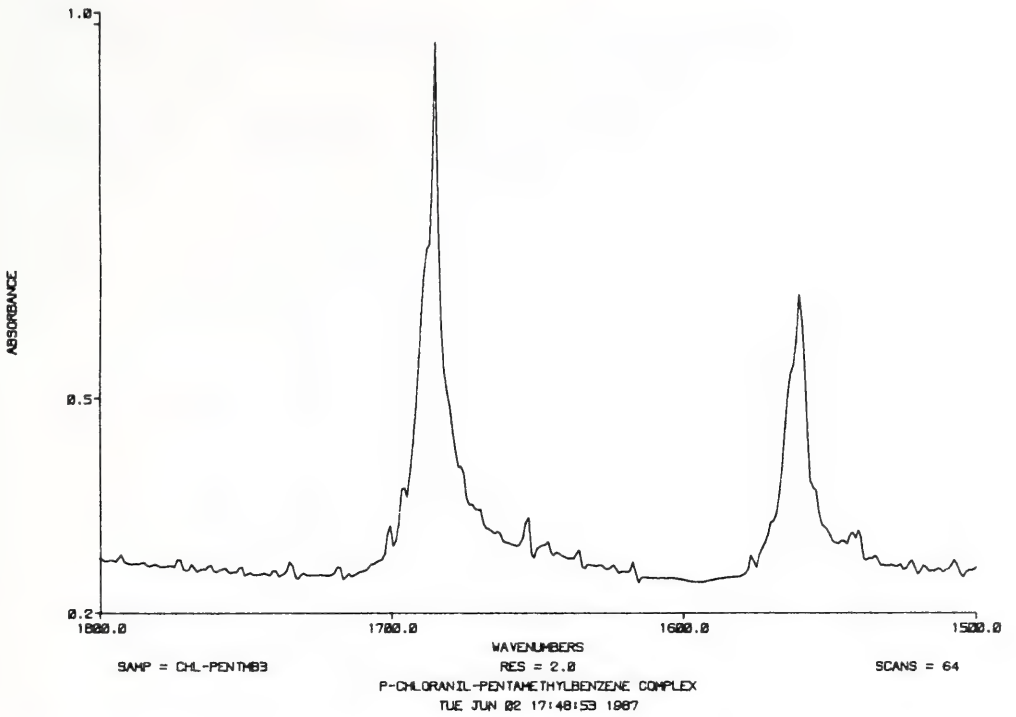

FIGURE 3. Spectra of Chloranil-Pentamethylbenzene Complex.

for chloranil and some selected complexes. The positions of the carbonyl vibrational absorptions and the shifts of these absorptions relative to the uncomplexed quinones are listed in Table I. The vibrational absorption positions are functions of the bond orders of the carbonyl bonds. The donation of electron density into the antibonding orbitals

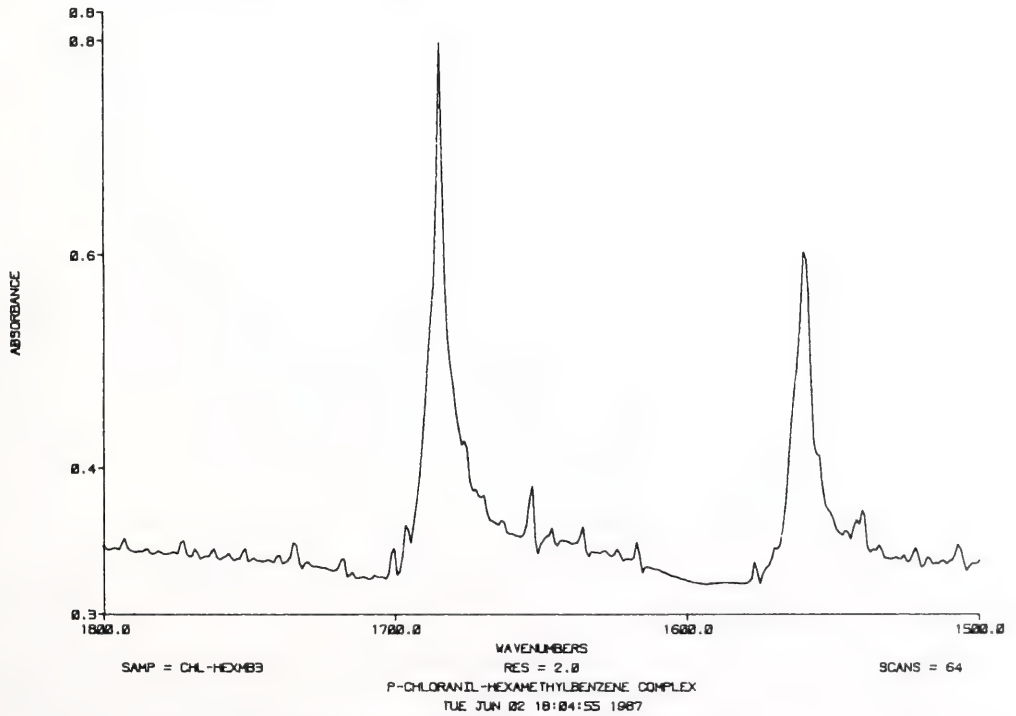

FIGURE 4. Spectra of Chloranil-Hexamethylbenzene Complex.

TABLE I. CO vibrational absorptions and absorptions shifts for tetrahalo-1, 4-benzoquinone charge-transfer complexes.

Complex(donor-acceptor)	$\nu_{CO}(CM^{-1})$	$\Delta_{CO}(CM^{-1})$
1,2,4,5-tetramethylbenzene-p-fluoranil	1695.5	4.9[a]
	1679.0	
pentamethylbenzene-p-fluoranil	1694.6	5.8[a]
hexamethylbenzene-p-fluoranil	1692.3	8.1[a]
tetrakis(dimethylamino)		
ethylene-p-fluoranil	1675.1	25.3[a]
1,2,4,5-tetramethylbenzene-p-chloranil	1689.6	0.0[b]
	1679.0	
pentamethylbenzene-p-chloranil	1684.9	4.7[b]
hexamethylbenzene-p-chloranil	1684.8	4.8[b]
tetrakis(dimethylamino)	1627.3	17.3[b]
ethylene-p-chloranil	1657.8	21.2
1,2,4,5-tetramethylbenzene-p-bromanil	1679.4	0.6[c]
pentamethylbenzene-p-bromanil	1678.5	1.5[c]
hexamethylbenzene-p-bromanil	1675.2	4.8[c]
tetrakis(dimethylamino)	1669.6	10.4[c]
ethylene-p-bromanil		

[a] The shifts are relative to the fluoranil CO vibrational absorption at 1700.4 cm^{-1}.
[b] The shifts are relative to the chloranil CO vibrational absorption at 1689.6 cm^{-1}.
[c] The shifts are relative to the bromanil CO vibrational absorption at 1680.0 cm^{-1}.

of quinone acceptors by the aromatic donors and the tetrakis(dimethylamino)ethylene during complexation reduces the bond order of the carbonyl bonds and causes their vibrational absorptions to shift to lower wavenumbers. The magnitudes of these shifts are related to the extent of electron donation into the antibonding orbitals of the quinone acceptors which in turn is related to the strengths of the donor molecules, the acceptor strengths of the quinones, and the stability of the charge-transfer complexes. In this study the following three issues were addressed: acceptor ability of the quinones and its dependence on the electronegativity of the halogen, donor strength of the aromatic and its relationship to the extent of ring alkylation, and the relative donor strenghts of the π aromatic donors and a σ amine donor.

Three tetrahalo-1,4-benzoquinone acceptors were used in this study, *p*-bromanil, *p*-chloranil, and *p*-fluoranil. For this series of acceptors the electronegativity of the halogen increases (Br-2.9, Cl-3.5 and F-4.0) causing more charge to be removed from the quinone ring and the acceptor strength of the quinone to increase. This increase in acceptor ability of the quinone allows more electron density to be accepted into its antibonding orbitals, and it results in a greater reduction in the carbonyl bond order and a greater shift to lower wavenumbers of the carbonyl infrared absorption. Examination of the spectral data in Table I shows that as a group the carbonyl shifts decrease going from *p*-fluornail complexes (4.9, 5.8, 8.1, 25.3 cm^{-1}) to chloranil complexes(0, 4.7, 4.8, 17.3 cm^{-1}) to *p*-bromanil complexes (0.6, 1.5, 4.8, 10.4 cm^{-1}).

The aromatic donors used in this study were 1,2,4,5-tetramethylbenzene, pentamethylbenzene, and hexamethylbenzene. In the complexes with these donors, the aromatic compound donates electron density from its π system to the quinone π antibonding orbitals. As the number of electron releasing methyl groups on the aromatic ring increases, the donor strength of the aromatic compound also increases (3, 5). The spectral data in Table I shows that the carbonyl shifts are largest for hexamethylbenzene and that they decrease as the number of methyl groups and the donor of the aromatic decrease.

Tetrakis(dimethylamino)ethylene is described in the literature as an extremely strong Lewis Base, a super donor (1). The large donor capacity of the tetrakis(dimethylamino)-ethylene is the result of the lone pair electrons on the highly alkylated amine groups. Our studies show (Table I) that sizeable carbonyl shifts (25.3, 17.3, 10.4 cm^{-1}) to lower wavenumbers exist in the complexes of the tetrakis(dimethylamino)ethylene with fluoranil, chloranil, and bromanil. This is in complete agreement with a large donation of electron density from the lone pair electrons on the tetrakis(dimethylamino)ethylene into the quinone π antibonding orbitals, a significant reduction of the quinone carbonyl bond order, and a large shift to lower wavenumbers of the quinone carbonyl infrared absorption.

Acknowledgments

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Infrared Studies of Charge-transfer Complexes of Tetracyanoethylene and Substituted Aromatics

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Introduction

The tetracyanoethylene molecule contains a highly conjugated π system which includes its carbon-carbon double bond and its four triply bonded cyano groups. The antibonding orbitals of the delocalized π system of the tetracyanoethylene can function as an environment to accept electron density from Lewis Base types of molecules. This transfer of electron density from the Lewis Base to the tetracyanoethylene forms a charge-transfer bond and results in the formation of a molecular association or charge-transfer complex (2). The charge-transfer bonds in these complexes are similar to the kinds interactions that characterize drug binding, enzyme activity, and other biologically interesting processes (7,8,9). It has also been postulated that a number of chemical reactions proceed through charge-transfer complex type of intermediates (1,2). The nature of the charge-transfer bond and stability of charge-transfer complexes is important to the understanding of the mechanism of these important chemical processes and has been the subject of a number papers in the literature (2).

Tetracyanoethylene forms charge-transfer complexes with substituted aromatic compounds. The sandwich structure which has been suggested in the literature for these charge-transfer complexes is pictured in Figure 1 (2,3,6). In these complexes the aromatic molecules

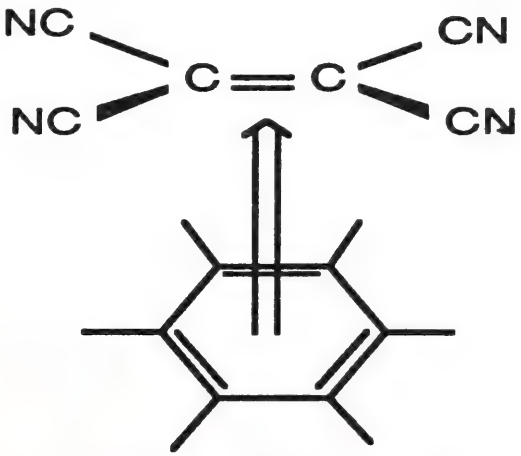

Figure 1. Tetracyanoethylene-aromatic Charge-transfer Complex

donate electron density from their π orbitals to the π antibonding orbitals of the tetracyanoethylene to form a charge-transfer bond between the aromatic donors and the tetracyanoethylene acceptor. This transfer of the electron density to the tetracyanoethylene antibonding orbitals causes the bond order of the cyano groups on the tetracyanoethylene molecule to decreased, and results in the decrease in the infrared vibrational frequency of the cyano bond. The magnitude of the decrease in both the bond order and the vibra-

tional frequency of the cyano group will be dependent on the extent of electron density transferred and the donor strength of the aromatic compound. In this study the charge-transfer complexes of tetracyanoethylene and substituted methylbenzenes (hexamethylbenzene, pentamethylbenzene, and 1,2,4,5-tetramethylbenzene) were prepared, and the location of the infrared absorptions of cyano stretching frequencies in the complexed tetracyanoethylene were determined. The magnitudes of the shifts to lower wavenumbers of the cyano stretching absorptions in the complexes relative to the cyano stretching absorption in uncomplexed tetracyanoethylene were determined and related to the donor strength of the aromatic compound.

Experimental

Each complex was prepared by dissolving tetracyanoethylene and the appropriate aromatic compound (hexamethylbenzene, pentamethylbenzene, and 1,2,4,5-tetramethylbenzene) in 1,2-dichloroethane in a one to five molar ratio and allowing the resulting solution to evaporate. The preparations were carried out in excess aromatic in order to use Le Chatelier's Principle to minimize the amount of uncomplexed tetracyanoethylene.

The infrared spectra was recorded on a Digilab FTS 40 Fourier Transform Infrared Spectrometer. The spectra of the complexes were collected at 2 cm^{-1} resolution in a KBr matrix. The complex concentrations in KBr were adjusted to be 2% by mass for each complex. Multiple sample preparations were carried out for each complex and the spectra recorded for each of these samples to determine the dependence of the position of the carbonyl absorption on the complex concentration in the KBr matrix. For the experimental variations in concentration of the complexes due to sample preparation, no more than a 0.2 cm^{-1} shift in the cyano absorptions was observed due to matrix effects.

Results and Discussion

Fourier transform infrared spectroscopy is characterized by its high sensitivity and its ability to determine accurately the positions of vibrational absorption bands. These characteristics make FT IR spectroscopy well suited for the study of the stability of the deeply colored charge-transfer complexes of tetracyanoethylene and substituted aromatic compounds. The vibrational absorptions of the cyano groups in these charge-transfer complexes are located in a region from 2500 to 2000 cm^{-1}. The spectra in this region of the tetracyanoethylene and its complexes with hexamethylbenzene, pentamethylbenzene and 1,2,4,5-tetramethylbenzene are shown in Figures 2-5. The positions of the cyano vibrational absorptions and the shifts of these absorptions relative to the uncomplexed tetracyanoethylene are listed in Table I. The positions of these vibrational absorptions are functions of the bond orders of these bonds. The donation of electron density to the antibonding orbitals of tetracyanoethylene by the aromatic donors during complexation recudes the bond order of the cyano bonds and causes their vibrational absorptions to shift to lower wavenumbers. The magnitudes of these shifts are related to the extent of electron donation into the antibonding orbitals of the tetracyanoethylene acceptor which in turn is related to the donor strength of the aromatic compound and the stability of the charge-transfer complex. Hexamethylbenzene with its six electron releasing methyl groups is the strongest donor in the series and exhibits the largest shift in its cyano vibrational absorption. The shifts for this series of complexes decreases as the number methyl groups on the ring and the donor strength of the aromatic decreases.

The shifts in the cyano vibrational absorptions are in agreement with other studies reported in the literature relating the equilibrium constant for the complex formation reaction and λmax of the charge-transfer band to the number of methyl groups on the aromatic ring, the donor strength of the aromatic, and the stability of the complex (4, 5). The literature values for K (equilibrium) for the complex formation reactions, λmax

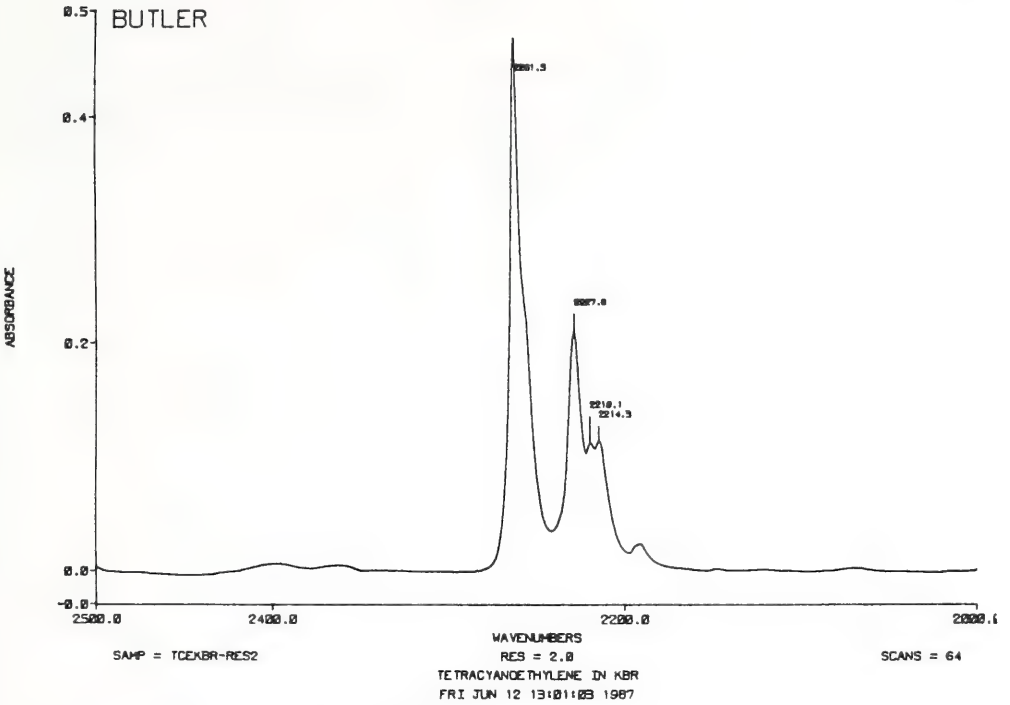

FIGURE 2. Spectra of Tetracyanoethylene

of the charge-transfer bands, and the cyano shifts from this study are listed in Table II. This data shows that as the number of methyl groups on the ring and the donor strength of the aromatic increases, the equilibrium constant for the complex formation reaction increases, λmax of the charge-transfer bands increases, the position of the cyano vibrational absorption decreases, and the shift of the cyano vibrational absorption relative to the uncomplexed tetracyanoethylene increases.

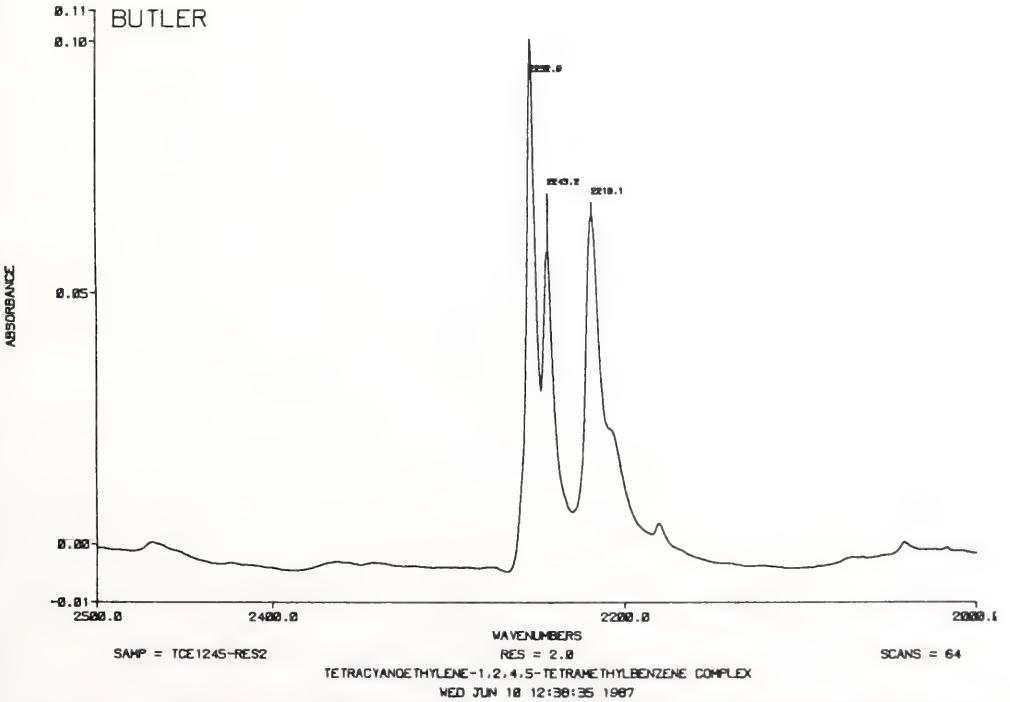

FIGURE 3. Spectra of Tetracyanoethylene—1,2,4,5-Tetramethylbenzene Complex

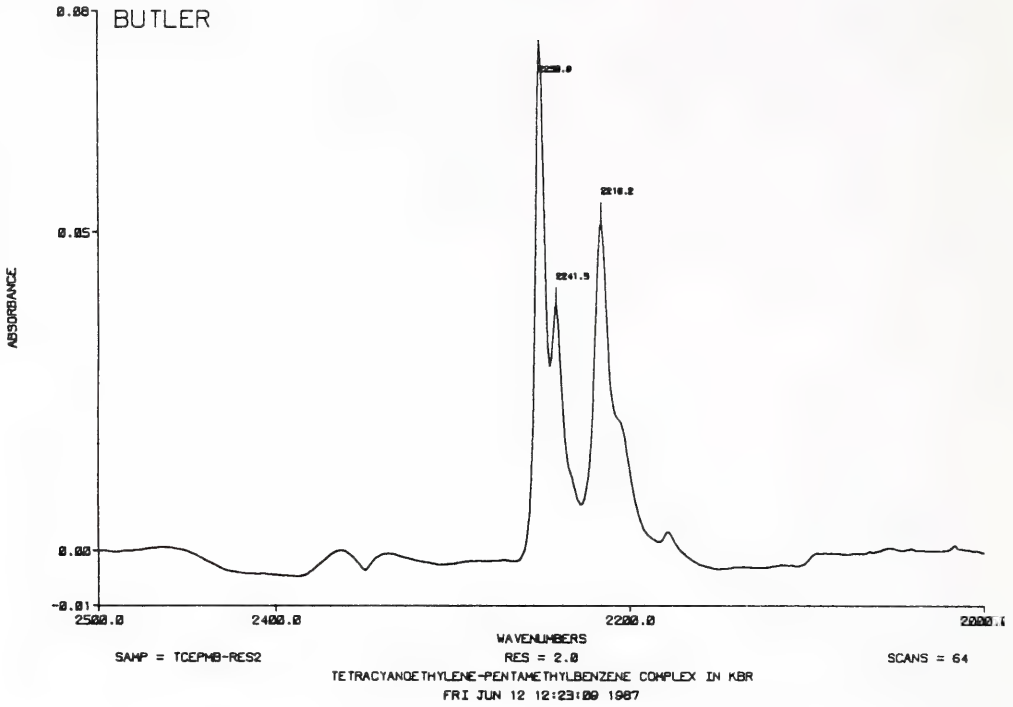

FIGURE 4. Spectra of Tetracyanoethylene-Pentamethylbenzene Complex

The authors wish to thank the Holcomb Research Institute and the Butler University Academic Grants Committee for the funding for this research project.

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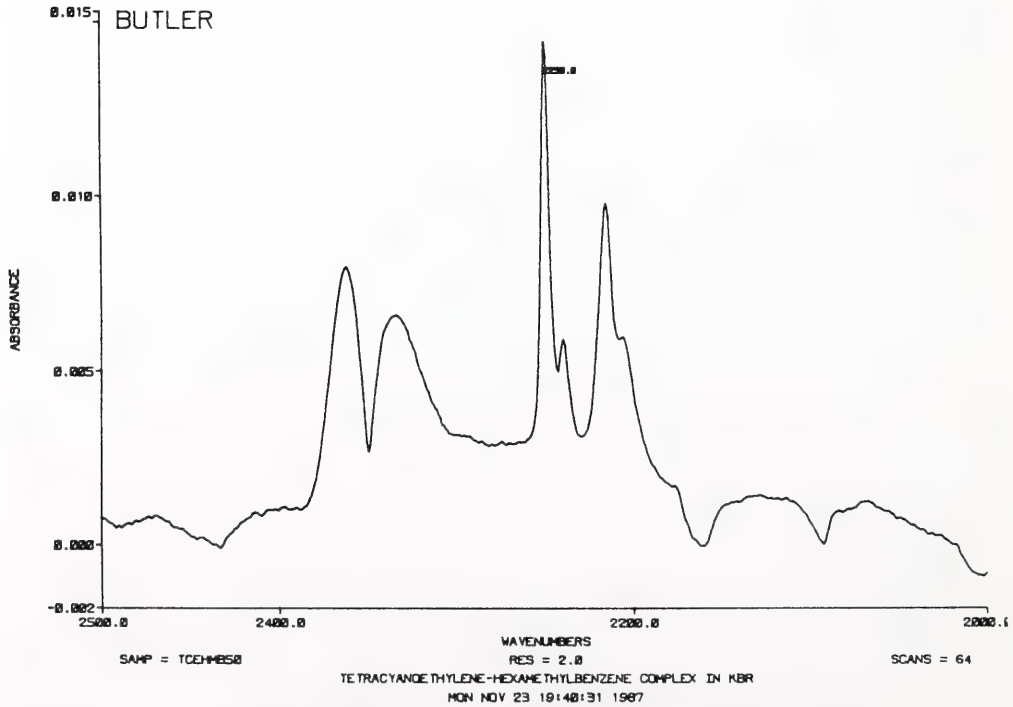

FIGURE 5. Spectra of Tetracyanoethylne-Hexamethylbenzene Complex

TABLE I. CN vibrational absorptions and absorptions shifts for tetracyanoethylene-aromatic charge-transfer complexes.

Donor	ν CN (CM^{-1})	$\Delta\lambda$ CN (CM^{-1})a
1,2,4,5-tetramethylbenzene	2252.9[b]	8.6
	2218.1	9.7
pentamethylbenzene	2250.9[b]	10.6
	2216.2	11.6
hexamethylbenzene	2250.0[b]	11.5
	2216.2	11.6

[a] The shifts are relative to the tetracyanoethylene CN vibrational absorptions at 2261.5 and 2227.8 cm^{-1}.
[b] The major CN absorption.

TABLE II. The CN vibrational absorption shifts and the literature values for K_{eq} and λmax.

Donor	$\Delta\nu$ CN (CM^{-1})a	K eq [b]	λ_{max}(nm)c
1,2,4,5-tetramethylben ne	8.6	1.15	480
pentamethylbenzene	10.6	4.36	520
hexamethylbenze	11.5	12.00	545

[a] The shifts are relative to the tetracyanoethylene CN vibrational absorption at 2261.5 cm^{-1}.
[b] ref. 8.
[c] ref. 9.

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The pH-potential Dependence of the Arsenic Electrode: A Preliminary Study

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Introduction

As a result of his electrochemical investigation of the galvanic couple,

Pt / H_2(g) / $HClO_4$(m) // $HClO_4$(m), As_2O_3(s) / As-Pt,

Schuhmann (9) reported a standard reduction potential of 0.234 volt at 25 °C. for the electrode reaction,

$$As_2O_3(s) + 6\ H^+ + 6\ e^- = 2\ As(s) + 3\ H_2O(1).$$

Arsenic behaves as a metalloid and like antimony and bismuth should function as a metal-metal oxide electrode and can be used to measure the pH of a solution potentiometrically. Moussa (7) used the arsenic electrode as the indicator electrode in the potentiometric titration of a dilute solution composed of a mixture of the acids, H_3PO_4, $HC_2H_3O_2$, and H_3BO_3, with NaOH. The titration was monitored with the aid of the electrochemical cell,

Pt-As / H^+ // satd. calomel electrode.

From these measurements he reports the reduction potential in volts for the arsenic electrode to be

$$E_{red} = 0.335 - 0.0597\ pH$$

at 25 °C., valid in the pH range 2.24-11.59. In both researches the arsenic electrodes were prepared by sublimation of the element onto a platinum wire electrode. In this study the electrodes were prepared by the electrodepostion of arsenic onto a base metal from a chloride plating bath.

Procedure

The arsenic electrodes were prepared by electrodeposition onto the base metal from an HCl-As_2O_3 bath (25 g As_2O_3 per liter of 7 M HCl) recommended by Menzies and Owen (6). The electrodes were plated with arsenic for 15 minutes at a current of 20 milliamperes at room temperature. The anode and cathode compartments were separated by a porous glass frit, a graphite rod served as anode, and the catholyte was stirred during the electrolysis. These conditions produced a bright adherent, metallic plate of elemental arsenic. Platinum wire (surface area 0.5 cm^2), platinum foil (surface area 2 cm^2), copper wire (surface area 0.8 cm^2), and copper wire bent into a spiral (surface area 3 cm^2) on which a bright copper deposit was electroplated served as base metal electrodes. The platium foil electrodes were the most stable. The copper plating bath used is described by Blum and Hogaboom (3). The bath composition is $CuSO_4.5\ H_2O$ g/l, H_2SO_4 15 g/l, thiourea 0.01 g/l and molasses 0.8 g/l; the plating was done at a current of 80 milliamperes for 20 minutes in a stirred bath with a copper wire anode.

All buffer solutions were prepared in CO_2-free distilled water from chemically pure reagents. All the reagents were reanalyzed by the methods described by Rosin (8). The pH values for the various buffer solutions were determined electrometrically with the aid of the following galvanic cell

Pt / H_2(g) / buffer // satd. calomel electrode.

The hydrogen electrode was a Hildebrand-type, and the saturated calomel electrode was a commerical, porous plug-type (Fisher Scientific Company 13-639-52). Six standard buffer solutions recommended by Bates (2) were used to determine the standard reduction poten-

TABLE 1: Primary Standard Buffer Solutions at 25 °C.

Buffer Solution	Molarity	pH
Potassium Tetroxalate	0.04962	1.679
Potassium Dihydrogen Citrate	0.04958	3.76
Acetic Acid	0.0100	4.718
Sodium Acetate	0.0100	
Potassium Dihydrogen Phosphate	0.02490	6.865
Sodium Hydrogen Phosphate	0.02490	
Potassium Dihydrogen Phosphate	0.008665	7.413
Sodium Hydrogen Phosphate	0.03032	
Sodium Tetraborate	0.009971	9.180

tial for the calomel electrode. They are listed in Table 1. Buffer solutions of other pH values were prepared from $HC_2H_3O_2$-NaOH mixtures in which the total acetate concentration was 0.02 molar, KH_2PO_4-Na_2HPO_4 mixtures in which the total phosphate concentration was 0.05 molal, and $Na_2B_4O_7$-$HClO_4$ of $Na_2B_4O_7$-NaOH mixtures in which the initial tetraborate concentration was 0.02 molal.

All electromotive force measurements were made at a temperature of 25.00 ± 0.05 °C. with an Orion Digital pH Meter (Model 801). A polarographic H-cell with a glass frit served as the electrolysis vessel. Unless otherwise stated, those buffers in contact with the arsenic electrodes were degassed with nitrogen gas for 15 minutes prior to making any potential difference measurements. Even though the data in Tables 2 and 3 are presented in order of increasing pH, the choice of any particular buffer was done in a random fashion.

TABLE 2: E.M.F. Values in Volts for the Cell, Pt / H_2(g) / buffer / As

pH	Pt-Cu-As foil	Pt-Cu-As wire	Cu-Cu-As spiral	Cu-Cu-As wire
1.679	0.2350	0.2439	0.2432	0.2445
3.530	0.3480	0.3687	0.3556	0.3326
3.776	0.3566	0.4053	0.4053	0.3363
4.032	0.3611	0.3823	0.3790	0.3503
4.044	0.4718	0.4741	0.4727	0.4729
4.351	0.4742	0.4776	0.4760	0.4768
4.718	0.4824	0.4846	0.4826	0.4704
5.102	0.4833	0.4850	0.4864	0.4879
5.410	0.4640	0.4646	0.4775	0.4470
6.280	0.4690	0.4752	(0.5374)	0.4796
6.865	0.4409	(0.5570)	0.4936	0.4562
7.413	0.4749	0.4658	0.4722	0.4596
7.572	0.4709	0.4761	0.4817	0.4818
8.917	0.4684	0.4714	0.4798	0.4757
9.180	0.4489	0.4573	0.5055	0.4656

TABLE 3: E.M.F. Values in Volts for the Cell, As / buffer // satd. calomel electrode

pH	Electrode 1.	Electrode 2.	Electrode 3.	Average
4.051	0.044	0.028	0.053	0.042
4.697	0.057	0.042	0.072	0.057
5.425	0.113	0.112	0.110	0.111
6.248	0.184	0.181	0.181	0.182
6.858	0.217	0.219	0.210	0.215
7.408	0.246	0.249	0.243	0.246
8.167	0.286	0.285	0.269	0.280
8.619	0.307	0.308	0.293	0.302
9.177	0.330	0.333	0.336	0.333

Results and Discussion

The saturated calomel electrode was calibrated on two separate occasions; separate preparations of the six buffers listed in Table 1 were used. For the galvanic cell,

Pt /H_2(g, p in atm.) / buffer // satd. calomel electrode,

the variation of the electromotive force with pH is of the form

$$E = A + B\,(pH + \tfrac{1}{2} \log p)$$

where A corresponds to the reduction potential of the saturated calomel electrode plus the average liquid-junction potential, B is the slope of the curve which is theoretically equal to 0.05915 volat at 25 °C., and p is the pressure of the hydrogen gas over the platinized platinum electrode in atmosphere that served as the hydrogen electrode. Least-squares treatment of the data in both cases gave a value of 0.2431 ± 0.0004 volt for A. In the first calibration the value for B was 0.05898 volt and in the second calibration the value of the slope was 0.05909 volt. In the determination of the value of the pH of the other buffers, the use of the experimentally derived values for A and B translates into an uncertainty of 0.006 pH unit.

If the arsenic electrode behaves as a reversible metal-metal oxide couple, then the electromotive force generated within the galvanic cell,

Pt / H_2(g) / buffer / As

will be constant over that pH range in which there is a unique electrode reaction at the arsenic electrode. Differing values for the electromotive force at differing pH values indicated a different electrode reaction. Table 2 summarizes the potential differences observed for four arsenic electrodes at various pH values. In Table 2, the designation "Pt-Cu-As foil" indicates that the electrode was prepared by the initial plating of copper followed by an arsenic plate onto a platinum foil electrode. The buffer solutions in contact with the arsenic electrodes were purged with nitrogen for 15 minutes prior to the measurements. Measurements of the electromotive force were recorded at 5, 10, and 15 minutes; the 15-min readings are listed in Table 2. The difference between the 5-min and 15-min values was less than 0.005 volt in the majority of cases. At all pH values the oxidation reaction occurred at the hydrogen electrode.

Excluding the first four buffers listed in Table 2, since the electrode reaction is obviously different from that observed in the buffer range, 4.044-5.410 ($HC_2H_3O_2$-$NaC_2H_3O_2$ buffers), 6.280-7.572 (KH_2PO_4-Na_2HPO_4 buffers), and 8.917-9.180 ($Na_2B_4O_7$-$HClO_4$ buffers) and the two spurious values enclosed in parentheses, the average reduction potential for the four electrodes is calculated as 0.474 ± 0.007 volt. The average of 0.242 volt at a pH of 1.679 approxmiates the value of 0.247 volt listed in "Standard Potentials in Aqueous Solutions" (1) for the reaction,

$$HAsO_2(aq) + 3\,H^+ + 3\,e^- = As + 2\,H_2O$$

The three buffers in the range 3.530-4.032 were formulated from potassium dihydrogen citrate and either perchloric acid or sodium hydroxide. The average value of 0.365 volt over the pH range covered by the citrate buffers might be attributed to an alternate reaction; however, when a potassium hydrogen phthalate buffer of pH 4.008 was used, the potential difference increased to a value above 0.45 volt. Glab (5) reports spurious potential values for the antimony electrode; consequently, a further investigation of the effect of oxalate on the potential of the arsenic electrode at various pH values is under study.

to measure the pH-potential relationship for the electro-deposited arsenice electrode, the potential difference developed in the galvanic cell,

Pt-Cu-As / buffer // satd. calomel electrode

was monitored with three separate electrodes. Three acetate buffers (pH range 4.051-5.425), three phosphate buffers (pH range 6.248-7.409), and three borate buffers (pH range 8.167-9.177) were employed. The buffer solutions were purged with nitrogen gas to remove dissolved oxygen before a potential-difference measurement was made. Preliminary ex-

periments indicated that a relatively stable potential difference was realized after the electrode had been immersed in the buffer for 15 min. In order to eliminate excessive corrosion of the arsenic by the solvent, the electrodes were stored in dry acetone when not in the buffer. The electrodes remained bright during the course of the measurements. The results are compiled in Table 3. In all instances the oxidation reaction occurred at the arsenic electrode.

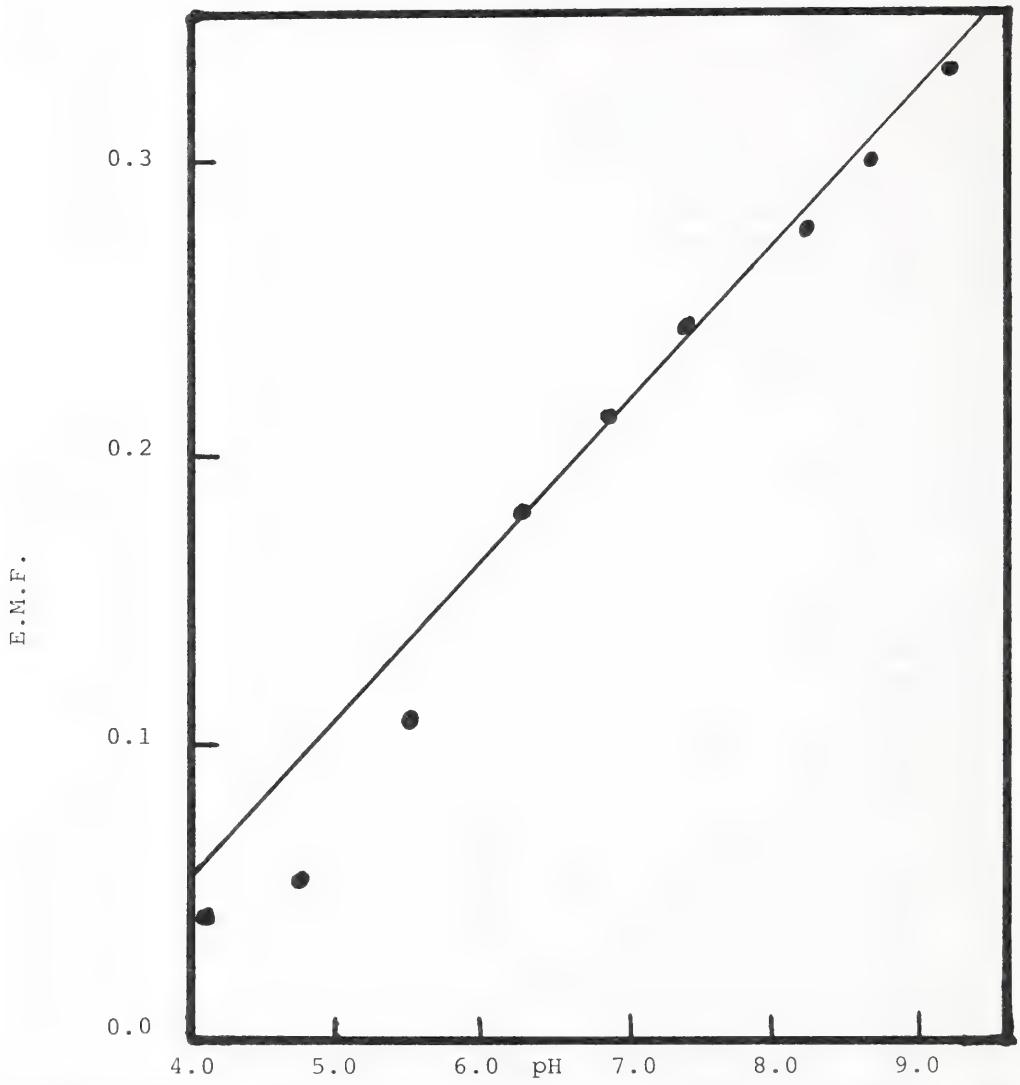

FIGURE 1. The pH-potential behavior for the galvanic cell, As / buffer // satd. calomel electrode, in the range 4.0 to 9.2.

The average potential difference is plotted as a function of pH in Figure 1. A linear relationship is observed over the pH range from 6.2 to 9.2. The least-squares fit to the data in this region yields the relationship in volts

$$E = -0.170 + .0556\ pH$$

This is the solid line on the graph. In this pH range the reduction potential for the arsenic electrode is calculated in volts as

$$E_{red} = 0.410 - 0.0556\ pH$$

In view of the experimental observation that in the cell,

$$Pt\text{-}H_2 \text{ / buffer / As,}$$

the acetate, phosphate, and borate buffers give similar values for the electromotive force,

no explanation can be given as to why the pH-potential behavior differs among these buffers. A non-Nernstian value of the slope is not surprising since metal-metal oxide electrodes are known to behave irreversibly. In a review of the pH-potential behavior of the antimony electrode Stock (10) summarizes the findings of 25 separate investigations; the reported slopes vary between 0.041 and 0.0647 volt, and in only 5 of the investigations is the theoretical slope of 0.0591 volt listed.

In order to examine the feasibility of using the aresenic electrode as the indicating electrode in potentiometric titrations, approximately 0.1 molar HCl, 0.1 molar $HC_2H_3O_2$, and 0.1 molar $HClO_4$ were titrated potentiometrically with approximately 0.1 molar NaOH. The base was delivered from a Sargent Constant Rate Buret Model G. The output from the potentiometer during the titration was fed into a recorder, Sargent Model SR, which was drived at a chart speed of one inch per minute. Solutions were stirred but not degassed during the titrations. Separate titrations of the same colume of each acid with the NaOH were monitored with the glass electrode/calomel electrode couple and with the arsenic/calomel electrode couple. The titration curves are reproduced in Figure 2. The behavior of the arsenic electrode during the titration of all of the acids

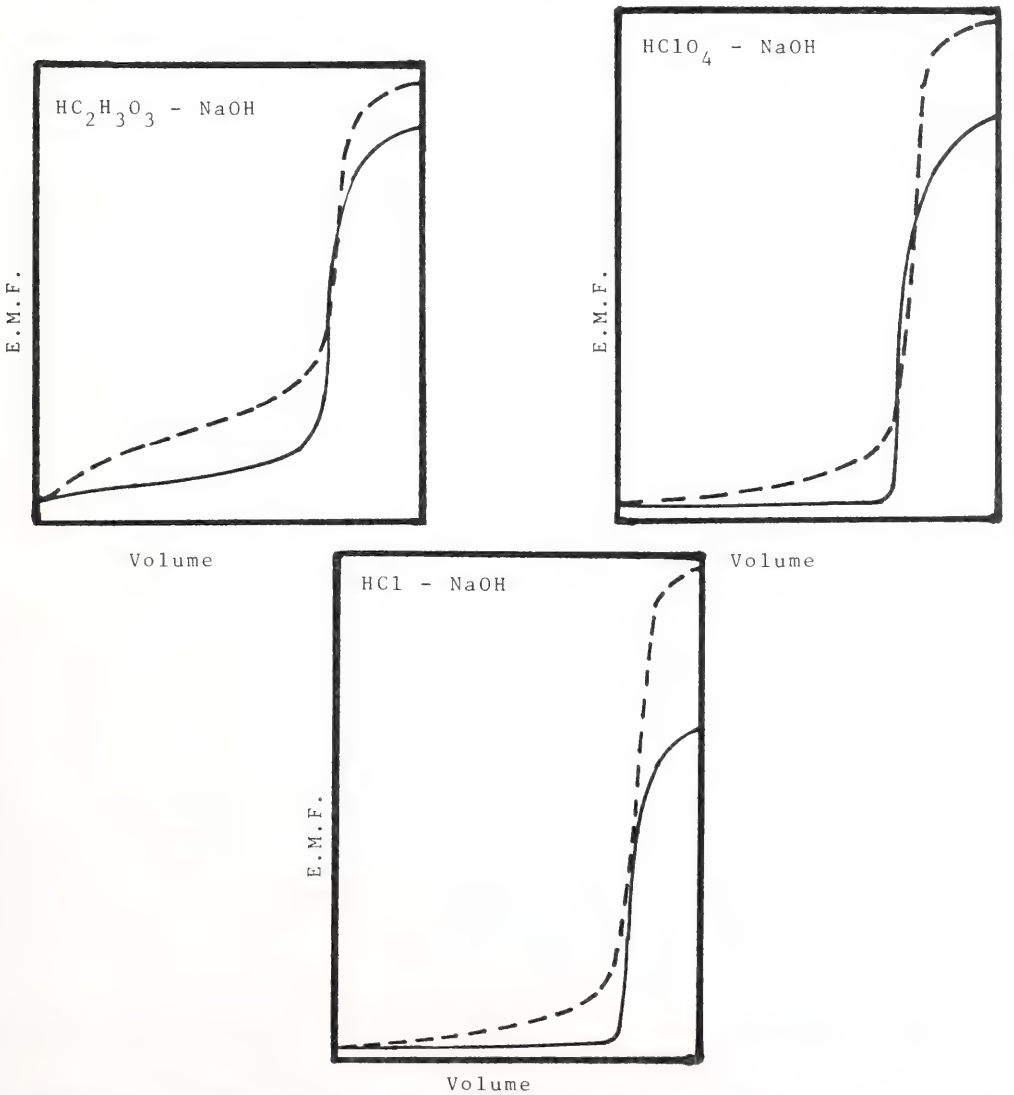

FIGURE 2. Titration curves using the galvanic cells, As / H^+ // satd. calomel electrode (—) and glass / H^+ // satd. calomel electrode (---).

is atypical in that little change in the value of the potential difference is noted until the equivalence point is reached. The volumes of NaOH used to reach the equivalence point with the aresenic and the glass electrodes were HCl (As-20.8 ml, glass-21.4 ml), $HClO_4$ (As-20.3 ml, glass-21.0 ml), and $HC_2H_3O_2$ (As-20.8 ml, glass-21.6 ml). The agreement is not good; however, the titrations were done on separate days. Since the difference seems constant, a constant error might be responsible. During an earler titration study using arsenic plated onto a platinum wire, the results were HCl (As-22.3 ml, glass-22.5 ml) and $HC_2H_3O_2$ (As-28.2 ml, glass-27.6 ml).

The conclusions reached concerning the behavior of the arsenic electrode are as follows:

1. The potential of electrodeposited arsenic does vary with pH; however, this potential is not a true reversible potential but a corrosion potential. The initial value of the potential at any pH is greater than the potential at the same pH when the electrode is stored in oxygen-free water overnight before the subsequent measurements are made. The potential of different electrodes approached a common value in each buffer after 12 to 24 hours. Similar behavior is reported by Edwall (4) for the antimony electrode.
2. The electrodeposited arsenic electrode is easily prepared and has a fast response to pH change; hence, it should find application as the indicating electrode in titrations in which the glass electrode cannot be used, in particular in certain non-aqueous systems.
3. The presence of dissolved oxygen in any buffer does affect the potential of the arsenic electrode. In a qualitative study we observed that the potential difference between the saturated calomel and arsenic electrodes is less than it is in an oxygen-free buffer. This effect is reversible in that, when the dissolved oxygen is removed with nitrogen, the potential difference increases. The magnitude of the difference seems to depend on pH; however, insufficient data are available to draw a definite conclusion as to whether the effect can be attributable to arsenic behaving as an oxygen electrode.
4. The electromotive force for the cell,

As / As_2O_3(s), buffer // satd. calomel electrode, was measured. The electrodes were stored in dry acetone between measurements. The $\triangle E/\triangle pH$ value for the three acetate buffers (pH range 4.02-5.40) was 0.027, for the three phosphate buffers (pH range 6.19-7.36) was 0.038, and for the three borate buffers (pH range 7.71-8.40) was 0.055. The potential difference values in both the phosphate and the borate buffers which were saturated with As_2O_3 were between 0.08 to 0.12 volt less than in the non-saturated buffers of approximately the same pH.

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ABSTRACTS

Factors Affecting Growth and Abundance of Young Walleye. Robert L. Ball, Indiana Department of Natural Resources, P.O. Box 16, Avoca, Indiana 47420.——An evaluation of growth and abundance of larval and juvenile walleye was conducted at Brookville Reservoir in east-central Indiana. Stocked larval walleye were collected by push-net, along with data on zooplankton abundance and limnological data at 5 stations for 3 years. Catch rates and survival of age 0 and 1 walleye were estimated from spring and fall shoreline electrofishing samples. Low water transparency reduced abundance of larvae at an uplake station. First year growth appeared to be related to water temperatures in May, with years having lower May water temperatures producing slow larval growth. Angler harvests of age 1 walleye dropped sharply as abundance of age 0 and 1 walleye increased. This was related to reduced first year growth due to increased intraspecific competition and low May water temperatures. Angler harvests of age 2 walleye were positively correlated with spring abundance of this age group.

Responses of Male Green Frogs (*Rana clamitans*) to Broadcasted Conspecific Calls. Jennifer J. Bucki and Stephen A. Perrill, Department of Biological Sciences, Butler University, Indianapolis, Indiana 46208.——A standard, single note mating call of *Rana clamitans* was repeatedly dubbed onto an experimental tape with 10 second intervals separating each call. Immediately after recording an unsolicited call from a subject, this experimental mating call was broadcasted to each subject from a distance of 50 cm. The subject's vocal response was recorded and other behaviors were noted. Analysis of the responses indicated no characteristic pattern for male green frogs. While the subjects' responses to the broadcasted calls were not predictable, all of the frogs' responses differed from their unsolicited behavior.

Stocking, Growth and Structural Changes over 20 years on Two Indiana State Forests. Burnell C. Fischer and David W. George, Department of Forestry and Natural Resources, Purdue University, West Lafayette, Indiana 47907, Ben Hubbard and Janet Eger, Indiana Division of Forestry, Martin State Forest, Shoals, Indiana 47581.——Continuous Forest Inventory (CFI) plots established in 1965 on Ferdinand State Forest—106 plots and Martin State Forest—88 plots, were located and remeasured in 1985 and 1986 respectively. Large increases in stocking indicate that these forests are rebounding from past misuse. Oaks continue to dominate the sawtimber-sized tree class but other species are increasing in importance. DBH growth trends follow those for other Indiana CFI data bases with yellow poplar and the red oaks greatest and hickories the slowest. Stand growth rates (net growth + ingrowth) are much

lower than those estimated by county soil surveys, Ferdinand = 97 bdft/ac/yr and Martin = 126, but compare favorably to other southern Indiana studies. Forest structure is uneven-sized reflecting both residual trees from previous high-grade cuttings and variable species growth rates.

Tree Species Composition Related to Aspect and Slope Position Following Clearcut Harvesting on the Hoosier National Forest. David W. George and Burnell C. Fischer, Department of Forestry and Natural Resources, Purdue University, West Lafayette, Indiana 47907.——There have been several studies on the response of tree regeneration after clearcutting in the central upland hardwoods region, but only limited analysis of species composition has been attempted. A large empirical data base (1801 plots) of the regeneration across an array of two easily attainable topographical factors, aspect and slope position, have been collected, and should relate to tree species regeneration. Data collected on the regeneration plots consisted of aspect, slope position and a tally of woody species in a dominant or codominant crown position. Species composition of an ordinal aspect and slope position matrix referred to as Upland Hardwood Site Quality Index (UHSQI) was divided into two age groups under 11 years and over 11 years (range to study 5 to 17 years) to identify any trends evident in the change of species composition. The UHSQI more clearly identifies tree species composition attributes of regenerated clearcuts on the Hoosier National Forest.

Nitrogen Fixation in Coarse Woody Debris. Paul C. MacMillan, Department of Biology, Hanover College, Hanover, Indiana 47243.——Preliminary results of acetylene reduction by samples of logs from Spring Mill State Park, indicate this is a potential source of increased N in coarse woody debris. Higher activity in maple and hickory wood and lower activity in oak wood, suggests an inhibition of N_2-fixation due to tannins in oak wood. Stimulation of acetylene reduction activity after incubation with a carbon supplement medium suggests the N_2-fixing bacteria were present in an inactive form in June. Higher activity at the soil-wood interface, than in other parts of the logs, suggests the N_2-fixing bacteria are from the soil, not from insect frass, fungal mycelia or plant roots. This study will monitor acetylene reduction activity in these same logs at 3-month intervals over a year.

Original Vegetation and Contemporary Landscape Patterns in Southern Elkhart County, Indiana. David J. Hicks and Marisa Yoder, Department of Biology, Manchester College, North Manchester, Indiana 46962.——US Land Office survey records from 1828 were analyzed to determine original vegetation patterns. Major vegetation types were beech-maple forest (occupied 78.8% of total area), oak-hickory forest (11.8%), dry prairie (3.7%), wetlands (3.4%) and floodplain forest (2.3%). Major land uses in 1978 were agriculture (78.1%), urban (8.3%), beech-maple woodlot (7.8%), floodplain and adjacent forests (4.2%), open water and wetlands (0.9%), and oak-hickory woodlots (0.7%). The average beech-maple woodlot had an area of 21.8 ha, and the average oak-hickory woodlot was 29.5 ha. Woodlots were randomly distributed with regard to each other and with regard to original forest type. Average distance between woodlots was 280 m.

Changes in the Potential Forage Fish Populations in Indiana Waters of Lake Michigan: 1973 Compared to 1984-86. Kevin J. McKeag and Thomas S. McComish, Department of Biology, Ball State University, Muncie, Indiana 47306.——The potential nearshore forage fish populations were sampled by bottom trawling at sites in Lake Michigan near Michigan City, Indiana from June through August of 1973 and 1984-86.

The total annual catch of adult fish, defined as age one or older, captured at combined sites was evaluated for the six major species composing the fish community.

The alewife (*Alosa pseudoharengus*) and trout-perch (*Percopsis omiscomaycus*) populations both declined dramatically at 71% and 87%, respectively, comparing 1973 to 1984-86. During the same period, yellow perch (*Perca flavescens*) increased 72 fold and bloater (*Coregonus hoyi*) increased over 3000 fold. By comparison, the spottail shiner (*Notropis hudsonius*) population was relatively stable and the rainbow smelt (*Osmerus mordax*) population fluctuated widely.

Significant changes have occurred in the near-shore fish community of extreme southern Lake Michigan in Indiana since the early 1970s. Although several factors may be involved, the major contributing factor is likely predator-prey interactions as a result of increased salmonid predator density.

Possible Mechanism of Female Choice in *Hyla Chrysoscelis*. MOLLY MORRIS AND STACIA YOON, Department of Biology, Indiana University, Bloomington, Indiana 47405.——A large male mating bias has been documented for a southern Indiana population of *Hyla Crysoscelis* in three out of four seasons studied. Preliminary observations suggest that female choice may have a significant influence on this non radom mating pattern. To explore the possible mechanisms on which females base their choice, both laboratory and field female discrimination experiments were conducted in relation to various parameters of the male's call and body size. In both the laboratory and the field experiments call rate is most important regardless of male size. However, when call rate is not variable frequency becomes significant.

Selected Metal Ion Concentrations in River Waters in Fulton, Kosciusco, Wabash and Whitley Counties. JAMES T. STREATOR AND ALLEN S. KRALL, Manchester College, North Manchester, Indiana 46962.——As part of a study of the quality of life in the four north eastern Indiana counties of Fulton, Kosciusko, Wabash and Whitley, the authors monitored the concentrations of five metal ions, pH and hardness in the three rivers that flow through these counties. Next summer, selected metal ion concentrations in standing bodies of water in these four counties will be monitored. Ions monitored included those of cadmium, chromium, copper, iron, potassium. Additionally, ions contributing to hardness, calcium and magnesium, and pH were monitored. The results and their significance will be presented.

A grant from the Eli Lilly Foundation provided funds for student work and for sample collection.

Seven Years of Change in Seedling Density Following Windthrow in an Old Growth Beech-Maple Forest in Northern Indiana. VICTOR RIEMENSCHNEIDER, Department of Biology, Indiana University at South Bend, P.O. Box 7111, South Bend, Indiana 46634.——Bendix Woods Nature Preserve, an old growth beech-maple forest, suffered extensive damage during severe windstorms on July 5, 1980. In May 1981, 100 random, 1.2m diameter plots were permanently marked and inventoried. All tree species less than one meter tall were counted each year in late April, May and September (1982-87). The average number of seedlings per hectare ranged from 15,208 in 1984 to 510,268 in 1985 with an average of 115,564. *Acer saccharum* and *A. nigrum* dominated the seedling level, ranging from 57 to 97% of total individuals. There was no significant difference in seedling numbers between plots inside and outside the windthrow area. The average number of seedlings per plot for maple and beech was greater outside than inside the damaged area. When maple was removed from total, there was a slight trend of increasing numbers with time. For a majority of species, the 100 random plots in the ca. 10 ha preserve were not a sufficient sample to determine seedling changes.

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Current Status of Natural Areas Listed by Lindsey, Schmelz and Nichols in *Natural Areas in Indiana and their Preservation.*

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Introduction

More than eighteen years have passed since the inventory of natural areas was completed by Lindsey et al. (1). This inventory was statewide in scope, and as such, was one of the first of its type in the nation. The inventory was funded by a grant from The Ford Foundation. In the preface, Gordon Harrison of The Ford Foundation noted that the inventory provided a basis for assigning protection priorities, helped to prevent destruction of natural areas through inadvertence, and was intended as a guide for those able to work toward saving some of what was left. In the years following publication, its significance has been demonstrated. Today, 18 years later, over half of the 155 areas listed in the study have at least some measure of protection, and only a handful have been destroyed.

Compilation

The files of the Division of Nature Preserves provided the data for this report. Information in these files came from years of field surveying by Division staff and many other natural area workers.

Results

Each natural area listed in the main body of Lindsey et al. (1) was categorized according to its current protection status. Highly protected areas (Table 1) include

Table 1. Natural areas that have some type of permanent protection. * = dedicated state nature preserve, # = partially protected only. DNR = Department of Natural Resources, NA = Natural Area, NNL = National Natural Landmark, NPS = National Park Service, TNC = The Nature Conservancy

	LINDSEY NAME	CURRENT NAME	COMMENTS
*	Ancient Pines Nature Area	Dunes	NNL
*	Barker Woods	Barker Woods	
*	Bear Creek Canyon	Portland Arch	NNL
*	Beckville Woods	Calvert and Porter	NNL
*	Beechwood Nature Preserve	Beechwood	
*	Bendix Gift Park Woods	Bendix Woods	
*#	Big Walnut Natural Area	Big Walnut	NNL
	Bird Marsh Nature Area	Bird Marsh Nature Area	State refuge
*	Blue Bluff	Blue Bluff	
	Blue River	Blue River	State scenic river
*#	Carnes Mill Site	Carnes Mill	Forest Service NA
*	Cedar Bluffs	Cedar Bluffs	
	Cowles Bog and Dunes	Cowles Bog	NPS, NNL

TABLE 1—Continued

	LINDSEY NAME	CURRENT NAME	COMMENTS
*	Donaldson's Woods	Donaldson's Woods	NNL
*	Ecology Coves	Dunes	NNL
*	Edna W. Spurgeon Reserve	Edna W. Spurgeon	
*	Fall Creek Gorge	Fall Creek Gorge	
*	Falls Canyon Nature Area	Rocky Hollow-Falls Canyon	NNL
#	Falls of the Ohio	Falls of the Ohio	Natl. Wildlife Cons. Area
*	Fern Cliff	Fern Cliff	NNL
*	Forty-acre Prairie	Beaver Lake	
*	Fox Island	Fox Island	
*#	Green Bluff	Green's Bluff	
*	Grider's Woods	Grider's Woods	
	Guthrie Woods	Guthrie Woods	TNC
	Hanging Rock	Hanging Rock	ACRES leased NNL
#	Hemlock Cliffs	Hemlock Cliffs	Forest Service NA
*#	Hemmer Woods	Hemmer Woods	NNL
	Hoot Woods	Hoot Woods	TNC, NNL
*	Jackson Woods	Laughery Bluff	
*	John Potzger Woods	Dogwood	
*	Laketon Bog	Laketon Bog	
*	Little Bluestem Prairie	Little Bluestem Prairie	
	Little Calumet River	Little Calumet River	NPS
*	Lloyd W. Bender Preserve	Lloyd W. Bender	
*	Lonidaw Nature Preserve	Lonidaw	
	Lubbe Woods	Lubbe Woods	DNR Nature Preserves
*	Marsh Lake	Marsh Lake	DNR Wetland Cons. Area
*	Merry Lea Nature Center	Merry Lea	partial ded.
*	Nasby Overlook Prairie	Mongoquinong—Nasby Fen	
*	New-Oak-Road Bog	Swamp Rose	
*	Olin Lake & Browand Woods	Olin Lake	
*	Pedestal Rock Nature Area	Pedestal Rock	
*	Pilot Knob Nature Preserve	Hayswood	
*	Pine Hills Nature Preserve	Pine Hills	NNL
	Pinhook Bog	Pinhook Bog	NPS, NNL
	Pioneer Mothers Memorial Forest	Pioneer Mothers Memorial Forest	Forest Service NA, NNL
*#	Prairie Border Nature Area	Tefft Savanna	
*	Rocky Hollow	Rocky Hollow-Falls Canyon	NNL
*	Spicer Lake	Spicer Lake	
*	Tamarack Bog	Tamarack Bog	NNL
*	Weaver Woods	Shrader-Weaver	NNL
*	Wesselman Park Woods	Wesselman Park Woods	NNL
#	Wing Haven	Wing Haven	ACRES owned, NNL
*	Woodland Bog	Woodland Bog	
	Wygant Woods	Wygant Woods	DNR Reservoirs NA
*	Yellow Birch Ravine	Yellow Birch Ravine	
*	Yellowwood Nature Area	Ogle Hollow	

dedicated nature preserves, designated scenic rivers, areas owned by the National Park Service, state and nationally designated refuges and conservation areas, areas owned by private conservation organizations, and U.S. Forest Service designated natural areas. Moderately protected areas (Table 2) include those whose owners have agreed to protect

TABLE 2. Areas with a moderate level of protection. R = Registered area, # = Partially registered only, NA = Natural Area, NNL = National Natural Landmark, TNC = The Nature Conservancy

	LINDSEY NAME	COMMENTS
	Allee Memorial Woods	Wabash College NA
	Big Four Cut, Wabash	NNL
R	Bluffs of Beaver Bend	
	Botany Glen, James S. Wilson Memorial Sanctuary	Marion College NA
	Bradford Nature Preserve	Indiana University NA
	Charles McClue Reserve	Steuben County NA
R#	Cypress Slough	
	Davis Forestry Farm, Compartment 1	Purdue University, NNL
	Ginn's Woods	Ball State University NA
R#	Goose Pond Cypress Slough	
R	Half Moon Woods	
R	Harrison Spring	NNL
	Hayes Nature Preserve	Hayes Arboretum
	John Cring Memorial Forest	Earlham College
R	Kado-lato Woods	
	Kramer Woods	willed to TNC, NNL
	Lilly-Dickey Woods	Indiana University NA
R#	Little Cypress Swamp Remnant	
R	Long Swamp Woods and Pond	
	Mary Gray Bird Sanctuary	Indiana Audubon
	McCormick's Cove	DNR State Park NA
R	Meltzer Woods	NNL
R	Mt. Pleasant Swamp	
R	Officers' Woods	NNL
	Parson's Swamp Woods	City of Goshen Parks & Rec
	Ross Biological Reserve	Purdue University
R	Rush Woods	
	Saint Mary's College Nature Area	Saint Mary's College NA
	Sedgwick's Rock Preserve	Earlham College
R	Shoemaker Bog	
R	Sourwood Site	
R	Spring Lake Woods and Bog	
	Sugar Creek	Proposed Scenic River
	Thornhill Nature Preserve of Huntington College	Huntington Collge
	W.S. Blatchley Sanctuary	Blatchley Nature Study Club

them as part of the Indiana Natural Areas Registry Program. Also included in this category are registered National Natural Landmarks (NNL) and areas owned and managed as natural areas by universities, colleges, and governmental agencies. Areas

still in need of protection are listed in Table 3. Sites considered to have acceptable

TABLE 3. Areas still in need of protection. DNR = Department of Natural Resources

LINDSEY NAME	COMMENTS
Alexander Woods	
Beaverdam Lake	DNR Fish and Wildlife
Biehle Oak Woods	
Cabin Creek Raised Bog	
Cataract Lake Spillway Cut	Geologic
Cave River Valley	
Cleveland Quarry	Geologic
Conboy Woods	
Disko High Bogs	
Fawn River Below Orland	
Flood Plain Beech-Maple Stand	
Hulman Park Woods	
Kentland Structural Anomaly	Geologic
Kickapoo Falls	
Manlove Woods	
Ogden Woods	
Old Dam, New Harmony Cut-off	Geologic
Rosbrugh Woods	
Three Glaciers Cut	Geologic
Umbrella Tree Ravine	
Walker Woods	
Winona Welch Botanical Area	
Wyland Lake	DNR Fish and Wildlife

status, either due to their nature or ownership, are shown in Table 4. Finally, sites that have either been destroyed or severely degraded since 1969 were placed in Table 5.

Discussion

A remarkably high number of the 155 areas listed in the inventory are today either highly protected (58 or 37%) or moderately protected (35 or 23%). Therefore, 60% (93) of the areas have at least some level of protection. Perhaps even more remarkable is the fact that only 11 sites have been destroyed (7%). Twenty-eight (18%) areas are considered to be secure areas. They are in no need of further protection, either due to their characteristics or ownership. It is important to note that of the 21 areas Lindsey et al. (1) considered to be "number one priorities", 15 have been permanently protected, and none have been destroyed.

This success is due mainly to two factors. During the survey, many of the landowners were contacted, making them aware of the specialness of their land. This insured that no areas were inadvertently destroyed. The work also served to motivate the preservation community, who set priorities and then concentrated their efforts on protection.

These protection efforts were aided by the passage of the Nature Preserves Act of 1967, which provided for the acquisition, management and permanent protection of nature preserves. Of the twenty areas that had been dedicated as state nature

TABLE 4. Natural areas that do not need further legal protection because of their ownership or site characteristics.

LINDSEY NAME
Ball State Wildlife Preserve
Barnes Swamp
Big Spring
Bluecast Woods
Cedar Marsh
Cold Friday Natural Area
Deam Oak Monument
Duning Woods
Elkhorn Falls
Gannon (or Stayner) Lake
Gene Stratton Porter State Mem. Woods
High Bridge Botanical Area
Honeywell Woods
Lane Lake
Lemon Woods
Lye Creek Prairie Burn
Merrilville Mire
Mystery Mounds
Purdue-Baker Wildlife Area
Reinking Memorial Forest
Shenk's Woods
Stayner Dry Prairie
Vermillion Falls
Wear Woods
White Squirrel Area
Williamsport Falls
Woods of the Giants
Wyandotte Spring

TABLE 5. Severely degraded or destroyed areas.

LINDSEY NAME
Brushy and Halfmoon Ponds
Cane Ridge
Caster's Woods
Clingenspell Woods
McColm Bog
Nurrenbern Woods
Quog Lake
South LaPorte Woods
Sword Moss Gorge
Tall Grass Sand Prairie
The James Bird Woods

preserves by January, 1972, 13 of these had been listed by Lindsey et al. (1). Work continued toward dedicating as many natural areas as possible, and today, 44 of the original 155 areas are dedicated nature preserves.

As positive as this seems today, at the time of publication, the destruction of a large number of sites seemed imminent. On October 2, 1970, Lindsey wrote to Schmelz "the rate of loss has now reached at least three tracts annually. . . There are not enough left to sustain this attrition rate much longer". Fortunately, because of this survey and the people who have dedicated their time to preserving natural areas, Lindsey's "premonition" was never realized.

There are still 23 sites (15%) left that need protection. Commitment is stronger than ever among preservationists to save these areas, and Lindsey's work is as important as ever as a motivator and guide.

Acknowledgments

The authors gratefully acknowledge Drs. Lindsey, Schmelz and Nichols for their important role in the field of natural areas preservation. William B. Barnes, first Director of the Division of Nature Preserves, is also acknowledged for his many years of hard work that resulted in the protection of so many areas. Finally, we are grateful to the many natural area workers who have continuted to pursue natural area protection and management.

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Coyote Vocal Responsiveness to Broadcast Auditory Stimuli in South Central Indiana

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Introduction

Coyotes (*Canis latrans*) were present in Indiana at the time of pioneer settlement, but were uncommon in most sections of the state until the 1960's (7). At that time, populations dramatically increased (8) and during the 1970's, total number of coyote pelts purchased by resident fur buyers increased 2-3x from one harvest season to the next. In the early 1970's, annual pelt sales were less than 100 but averaged over 2,000 by the end of the decade (6).

Coyote harvest data are useful indices of coyote distribution and abundance, but total harvests are strongly influenced by shifts in hunting and trapping pressure that may result from fluctuations in pelt values (4). Alternative means of obtaining measures of coyote populations are necessary for management purposes.

Aerial counts, scent-station surveys, and broadcasts of electronic siren wails or coyote-howl recordings to detect coyotes have been reported for some western states (1, 2, 3, 11, 12). The use of electronic siren wails, tape recorded wolf (*Canis lupus*) howls, and human howling to elicit coyote vocalizations have also been attempted in the Northeast (5, 10). This paper reports on the frequency of coyote vocalizations elicited through the use of playbacks of cassette-recorded siren wails, coyote howls, and train whistles from June 1986 through May 1987 in south-central Indiana.

Methods

Four test routes were established in portions of Lawrence, Orange, and Martin counties (Figure 1). Nine listening points (stops) were established at approximately 3.2-km intervals along each test route utilizing topographic features conducive to listening efficiency. Testing design was established to evaluate seasonal vocal responsiveness of coyotes to each of the three auditory stimuli. The order in which each test route was conducted was randomly selected each season with each route tested three times per season. Stimulus-type selection was also randomly established for each route test with each stimulus broadcast three times along each nine-stop route. Seasonal test intervals were spring (March, April, May); summer (June, July, August); fall (September, October, November); winter (December, January, February). Stimulus broadcasting was conducted as a two-trial test at each stop. A twenty-second segment of the cassette-taped stimulus was broadcast followed by a two-minute listening period, then rebroadcast and followed by a one-minute listening period. A positive response was recorded when one or more coyotes vocalized during either, or both, listening intervals.

Testing began from 30-60 minutes after sunset and was completed within 90 minutes. Tests were scheduled at the rate of one route per week, but adverse weather patterns necessitated occasional departure from this schedule.

Broadcasts of the auditory stimuli were through a portable cassette-tape player linked to a Model S-610 Perma Power Half-Mile Hailer 12-watt speaker manufactured by Perma Power Electronics, Inc. of Chicago, Illinois.

Log linear analysis of categorical data were initially used to test interaction be-

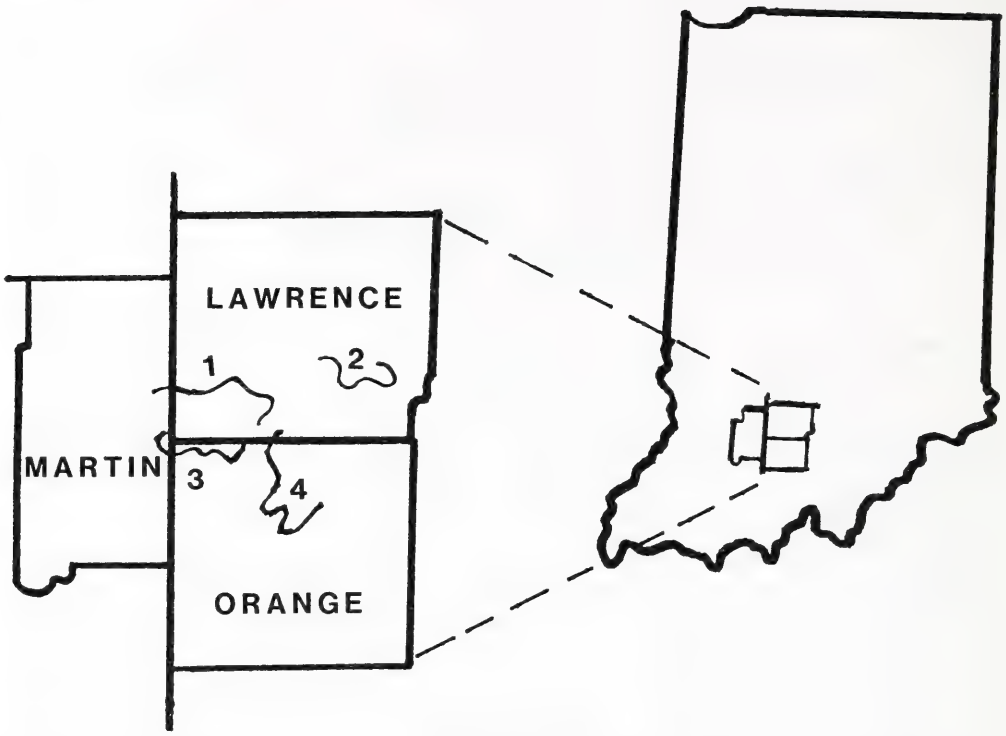

FIGURE 1. Locations of coyote vocal response test routes in south-central Indiana: Huron Route (1), Lawrenceport Route (2), Scarlet Route (3), Orangeville Route (4).

tween season and stimulus effects. Chi-square tests were calculated to evaluate significance of the main effects.

Results

One or more coyote group vocalizations were heard following 26 (6%) of the 432 broadcasts of the auditory stimuli (Table 1). Most elicited vocalizations (65%) followed tests of the coyote bark-howl and group yip-howl stimulus. Twenty-seven per cent of elicited vocalizations followed playbacks of the siren wail stimulus, and only 8% of the elicited vocalizations followed tests of the train whistle recording. Variation in vocal response levels among the three types of auditory stimuli were significant ($P < 0.001$).

Significant variation in coyote responsiveness ($P < 0.05$) occurred among the four seasons (Table 1). More responses (46%) were obtained in summer tests than in the fall (27%), spring (15%), or winter season (3%). The number of coyote vocalizations obtained among the four test routes was quite uniform. Overall response rates ranged from 4.6-6.5%, and variation in response levels among the routes was not significant ($P > 0.90$). Vocal responses were too few to permit evaluation of whether coyote responsiveness to stimulus type was consistent across seasons.

Discussion

Although the overall vocal response rate (VRR) by coyotes to broadcasts of the three types of auditory stimuli of 6% was low, the summer VRR of 22% (Table 1) to the coyote howl stimulus is comparable to the VRR of 26% following 1,286 siren soundings in the months of June through August over a three-year period in Iowa (2). It also compares favorably with the VRR of 25% documented for 14 radio-

TABLE 1. Seasonal responsiveness of coyotes to three types of auditory stimuli along four south-central Indiana routes from June 1986 through May 1987. Thirty-six tests were conducted for each stimulus type each season for a total sample size of 432.

Stimulus Type	Number Of Group Responses	Response Rate (%)
Summer		
Coyote Howl	8	22.2
Siren Wail	2	5.5
Train Whistle	2	5.5
	12	11.1
Fall		
Coyote Howl	3	8.3
Siren Wail	4	11.1
Train Whistle	0	0.0
	7	6.5
Winter		
Coyote Howl	3	8.3
Siren Wail	0	0.0
Train Whistle	0	0.0
	3	2.8
Spring		
Coyote Howl	3	8.3
Siren Wail	1	2.8
Train Whistle	0	0.0
	4	3.7
All Seasons		
Coyote Howl	17	11.8
Siren Wail	7	4.9
Train Whistle	2	1.4
	26	6.0

telemetered coyotes following siren soundings and human howling in the Adirondack Mountains of New York during August to mid-October (9). The overall VRR in this study is similar to the average response rate (7%) of coyotes to taped wolf howls and siren wails throughout the year in another New York study (5).

Responsiveness of coyotes to broadcasts of the auditory stimuli in this study may have been higher than the data presented indicates. There were instances where as many as three identifiable coyote groups were heard vocalizing after broadcasts of the siren wail or coyote howl recording at one listening site. However for statistical analyses, multiple group vocalization data were not included because additional group vocalization may have been in response to the elicited vocalization rather than to the broadcast stimuli. A total of 35 coyote group vocalizations was recorded, 26 of which occurred within the summer and fall seasons.

The train whistle stimulus had little merit in elicting coyote vocalizations. However, the tape recorded train whistle used, may have been of a lower frequency and intensity than train engine whistles in use at sites where coyote vocalizations have been reported to occur. It also may be that coyotes whose home ranges fall within proximity to railroad crossings are stimulated to howl only after they're exposed to a number of train whistle blasts and vocalizations reported by others may have been conditioned responses.

Use of broadcast auditory stimuli appears to be most valuable for determining coyote occurrence within a proposed area. Broadcasts of either the siren wail or a coyote howl recording are satisfactory to detect coyote presence in Indiana. Use of broadcast auditory stimuli during the summer or fall season seems most efficient for rapidly determining if coyote family groups might be present in a defined area. In two instances, coyote pup groups vocalized from their den site in response to the coyote howl recording during April testing. Concentrated efforts to broadcast either the siren wail or coyote vocal recording during the spring season might be useful in locating and obtaining pups for a particular project.

Acknowledgment

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Evidence for Potential Impact of Acid Deposition on Some Surface Waters of Extreme South Central Indiana in the Hoosier National Forest

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Introduction

Water quality evaluation of ponds in Perry and Crawford Counties of southern Indiana during fish population assessment revealed the possibility of impact from acidic precipitation. The low alkalinity concentrations found had not been previously reported for Indiana surface waters. Since several of the ponds had alkalinities within the known "sensitive" (6) range for impact from acid deposition, microcosm experiments using simulated acid rain (SAR) from a representative pond were completed to assess the potential for impact.

Simulation of ecosystem stress due to increasing acidic input was attempted in microcosm experiments using water treated with SAR and water with sediment treated with SAR. The models simulated water basins where one-third of the volume was replaced by acidic precipitation in the form of SAR having pH levels of 5.6, 4.5, 4.0 and 3.5. The characteristics evaluated with addition of SAR included alkalinity, conductivity, and pH.

The major objectives of this study were to classify a representative assemblage of south-central Indiana ponds and lakes for potential susceptibility to acid precipitation and to assess potential changes of those waters when treated with addition of acid precipitation in laboratory microcosm experiments.

Study Area

Ponds and lakes surveyed for susceptibility are located in the Hoosier National Forest of extreme south-central Indiana, from near the Ohio River in Perry County northward to central Crawford County. They are all man-made impoundments that receive runoff water from adjacent drainage areas and are situated in a variety of topographic locations from hilltops to hillsides and lowlands.

Oriole Pond, where water and substrate was collected for microcosm experiments, is located near State Route 66 in Perry County about 5 mi. north of the Ohio River. When sampled in 1985, the pond had an estimated surface area of 1.1 hec., a maximum depth of about 5 m, and an average depth of about 2.8 m. Oriole Pond water and substrate was used in microcosm experiments since it is a representative aquatic system in the survey area. In addition, the soils and basic geologic characteristics appear to be the type that could be conducive to surface water quality changes from acid precipitation. The soil in the pond vicinity was Typic Fragindalfs, fine silty, mixed and mesic (7). This soil is commonly known as Zanesville Silt Loam belonging to the Zanesville series of classification. It developed in silty loess, 18 to 48 in. thick, over material weathered from acid sandstone, siltstone and shale known to have a low buffering capacity.

Methods and Materials

Oriole Pond water samples and bottom sediment were collected in September from the pond surface at a point near the shoreline in about 0.5 m of water. Clean

glass containers were used to transport the samples to the laboratory where they were stored at room temperature in opaque boxes until experiments were initiated.

In the laboratory, microcosm experiments were conducted in two groups with the following experimental conditions:

1. Pond water alone with four treatment levels of SAR: pH 5.6, 4.5, 4.0, and 3.5
2. Pond water and sediment with four treatment levels of SAR: pH 5.6, 4.5, 4.0, and 3.5

Four replicate microcosms were used at each pH treatment level of SAR for the two groups resulting in a total of 32 microcosms; 16 with pond water alone and 16 with pond water and sediment.

Each of the 32 microcosms consisted of 250 ml of pond water placed in a clean 950 ml wide-mouth glass jar. In addition, 16 microcosms received 20 ml of substrate sediment. The four microcosm replicates for each SAR test level were aligned on a large table in the laboratory directly beneath a florescent fixture with two 48-in. cool-white tubes. Each microcosm was covered with a double layer of colorless translucent plastic to minimize evaporation. The photoperiod cycle used was 12 hr. of light followed by 12 hr. of darkness. All microcosms were established on December 19, 1985. They were left undisturbed until SAR experiments were initiated on February 7, 1986. This allowed a time period for establishment of water-sediment equilibruim and clearing of turbidity in microcosms. Water in all microcosms was clear and observed to be essentially colorless at the start of SAR experiments. The laboratory temperature ranged from 18 to 24 C over the total period from establishment of microcosms through SAR treatment and testing.

Stock SAR solutions were prepared using concentrated reagent grade sulfuric acid diluted with double-distilled water to pH 5.6, 4.5, 4.0 and 3.5. They were stored in glass bottles in the refrigerator prior to use. All pH determinations were made using a model 265 Instrumentation Laboratory pH meter read to the nearest 0.1 pH unit. Meter readings were temperature corrected and standardized using a pH 4.0 buffer solution.

Total alkalinity concentration of water was determined in field sampling and the laboratory using the standard titrametric procedure outlined in Standard Methods for the Analysis of Water and Wastewater (1). All field collections of water were from just beneath the surface in the middle of the lake or pond using a clear 300 ml glass stoppered bottle. Conductivity was measured using a model 33 Yellow Springs Instrument conductivity meter with temperature correction.

At the start of the SAR experiments on February 7, 100 ml of water was withdrawn from each microcosm using a clear syringe. The water was tested to establish the beginning levels of alkalinity, conductivity and pH. Next, 75 ml of SAR was added to each microcosm at appropriate pH levels using a clean syringe. Thus, each microcosm received SAR in a 1:2 ratio representative of a theorethical model where one-third of the volume was renewed by addition of stimulated rainfall to each microcosm system. Tests for alkalinity, conductivity, and pH were completed the day after SAR was added to each microcosm.

Results and Discussion

Field Survey of Surface Waters

A total of 21 ponds and lakes in Perry and Crawford counties were surveyed during June or July of 1983-86 (Table 1). Sizes of these impoundments range from 0.1 to 1.1 hectares. Classification of the lakes into two general categories was completed

TABLE 1. The names, classification, and locations of 21 ponds and lakes surveyed in the Hoosier National Forest in Perry and Crawford Counties, Indiana.

Name/Class*	County	Legal Description
Atkinson	Crawford	NW 1/4, SW 1/4, Sec 7, T 3 S, R 1 E
Deer	Perry	NW 1/4, NE 1/4, Sec 3, T 7 S, R 2 W
Derby*	Perry	NW 1/4, NE 1/4, Sec 6, T 6 S, R 1 W
Deuchars	Crawford	NW 1/4, NW 1/4, Sec 20, T 4 S, R 1 E
Gatchel*	Perry	E central, NE 1/4, Sec 29, T 5 S, R 2 W
Gerber*	Perry	NW 1/4, SE 1/4, Sec 27, T 6 S, R 2 W
German Ridge	Perry	N central, NE 1/4, NE 1/4, Sec 35, T 6 S, R 2 W
Harding*	Perry	SE 1/4, SW 1/4, Sec 6, T 6 S, R 1 W
Helwig Hollow*	Perry	N central, SW 1/4, Sec 27, T 6 S, R 2 W
Hemlock*	Crawford	SE 1/4, NE 1/4, Sec 8, T 3 S, R 1 W
Middle Deer*	Perry	NW corner, SE 1/4, NE 1/4, Sec 4, T 6 S, R 2 W
Oriole	Perry	SE 1/4, NW 1/4, Sec 11, T 4 S, R 1 W
Popcorn*	Crawford	SE corner, SW 1/4, NE 1/4, Sec 20, T 4 S, R 1 E
Range	Perry	W central, NE 1/4, Sec 20, T 5 S, R 2 W
Spring	Crawford	Center, NE 1/4, NE 1/4, Sec 30, T 3 S, R 1 E
Timberlake*	Perry	SE 1/4, NE 1/4, Sec 18, T 6 S, R 2 W
Triumph*	Perry	NE 1/4, NW 1/4, Sec 9, T 4 S, R 2 W
West Fork "A"	Crawford	SE corner, NW 1/4, SW 1/4, Sec 22, T 3 S, R 1 W
Wheatley*	Perry	SW corner, SE 1/4, SE 1/4, Sec 17, T 5 S, R 2 W
Whispering Pines	Crawford	Center, SW 1/4, NE 1/4, Sec 9, T 3 S, R 2 W
Unnamed	Perry	NW corner, NE 1/4, SW 1/4, Sec 2, T 4 S, R 2 W

* Ponds and lakes classified as sensitive to acid precipitation had measured total alkalinities equal or less than 10 mg/1. Additional information is available in unpublished Final Project Reports by Mc Comish (2,3,4) and Mc Comish and Kiley (5) on deposit in Braken Library at Ball State University.

on the basis of total alkalinity measurements which ranged from 6 to 128 mg/l. According to Omernik and Kinney (6) "it is generally agreed that waters of total alkalinity >200 ueq/1 (> 10 mg/l) are relatively insensitive to acidic deposition." A total of 11 of the lakes and ponds sampled were classified as "sensitive ($\leq$ 10 mg/l)", having total alkalinity values ranging from 6-10 mg/l with the mean at 7 mg/l (SD $\pm$ 2). The remaining 10 lakes and ponds were in the insensitive range (> 10 mg/l) with alkalinity values from 13-128 mg/l with the mean at 39 mg/l (SD $\pm$ 39). The survey data show over half (11 of 21) of the ponds and lakes classified as potentially sensitive to acid precipitation.

The high percentage of sample ponds and lakes in the sensitive classification appears representative of the impounded surface waters (natural lentic systems of any size are nonexistant) of the Hoosier National Forest area in Perry and Crawford Counties. This conclusion is justified on the basis of the variety of topographic locations and the wide distribution of the impoundments sampled within the defined area. A future comprehensive sample of south-central Indiana surface waters may provide a more exact estimate of the pond and lake numbers, locations, and degree of sensitivity to acidic deposition.

Microcosm Comparison Before SAR Treatment

The total alkalinity, conductivity and pH of microcosms with water alone were compared to those with water and sediment just prior to SAR treatment on February 7. Great differences were found in the characteristics evaluated using a *t*-test of means. The mean total alkalinity of water alone was 59.2 mg/l (SD $\pm$ 2.5) compared to water with sediment at 0.9 mg/l (SD $\pm$ 0.8) (Significant at $P < 0.001$). The mean pH of water alone was 7.9 (SD $\pm$ 0.1) compared to water with sediment at 5.7

(SD ± 0.2) (Significant at $P < 0.001$). The difference in conductivities of the two microcosms was not as great with the mean at 97.6 (SD ± 0.7) for water alone and 104.2 (SD ± 3.4) for water with sediment (Significant at $P < 0.001$).

These data indicate that the substrate material was responsible for significantly lowering both total alkalinity and pH while slightly increasing conductivity. The exact mechanisms involved are unknown but thought related to the acid sandstone, siltstone and shale predominant in soils of the area (7). As expected, these data reveal the importance of the lake basin, with inherent geologic and edaphic characteristics, in determining the nature of water quality components of surface water.

Pond Water Alone Microcosm Experiment

Microcosms with water only were treated with SAR at levels of pH 5.6, 4.5, 4.0, and 3.5 (Table 2). Since the pH of rain is naturally about 5.6, it was considered

TABLE 2. Comparison of selected water quality characteristics for experimental microcosms with water alone and water with substrate sediment after SAR treatments.

Microcosm/ Characteristic	SAR pH Treatment Levels							
	5.6		4.5		4.0		3.5	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Water Alone:								
Total Alkalinity (mg/l)	44.4	4.3	40.8	1.6	35.2*	4.4	26.3*	1.0
Conductivity (Micromohs)	69.0	7.9	59.8*	4.0	61.8*	2.2	64.3	2.6
pH	8.0	0.2	7.8	0.2	7.6*	0.8	7.7*	0.8
Water With Sediment:								
Total Alkalinity (mg/l)	0.7	0.8	1.4	0.9	0.6	0.5	0.2	0.0
Conductivity (Micromohs)	82.8	7.5	70.3*	9.2	80.5	3.1	76.5	1.7
pH	5.9	0.3	5.8	0.5	6.3	1.6	5.1*	1.0

* Indicates *t*-test of mean significantly different ($P < 0.05$) compared with SAR pH 5.6.

the baseline impact on water quality components. Therefore, SAR at pH 5.6 was used as a basis of comparison of measured changes. Total alkalinity ranged from 44.4 mg/l at SAR 5.6 to a low of 26.3 mg/l at SAR 3.5. The total alkalinity level at SAR 5.6 compared to both SAR 4.0 and 3.5 was significantly ($P < 0.05$) different. Conductivity change was less clear and more minor with SAR 5.6 at 69 micromohs compared to 60-64 at other SAR treatment levels. Differences in conductivity were significant comparing SAR 5.6 with SAR 4.5 and 4.0 ($P < 0.05$) but not significant with SAR 3.5. The comparison of pH revealed a gradual decreasing trend from 8.0 at SAR 5.6 to 7.7 at SAR 3.5. Comparison of SAR treatment levels with SAR 5.6 revealed significant ($P < 0.05$) differences at SAR 4.0 and 3.5.

The addition of SAR to microcosms results in a clear decrease of both total alkalinity and pH. Total alkalinity change from the level found with normal rainfall (SAR 5.6) resulted in a mean 8% lower at SAR 4.5 (Not Significant), 21% lower at SAR 4.0 (Significant at $P < 0.05$) and 41% lower at SAR 3.5 (Significant at $P < 0.05$). The change in pH from that in normal rainfall was less dramatic ranging from a 0.2 pH unit decrease at SAR 4.5 (Not Significant) to 0.4 and 0.3 pH units at SAR 4.0 and 3.5, respectively (Significant at $P < 0.05$). The addition of acid in the form of SAR had a progressive effect on buffering with the greatest effect at the lowest pH (3.5). Conductivity appeared to be reduced but results were less definitive than for total alkalinity and pH.

This experiment revealed that the water was significantly impacted with the addition of SAR especially at pH 4.0 and 3.5. The buffering capacity of the water was very limited, therefore it had little ability to accept the SAR without a resultant major change in basic chemical character.

Pond Water With Sediment Microcosm Experiment

Microcosms with water and sediment were treated with SAR at levels of pH 5.6, 4.5, 4.0, and 3.5 (Table 2) in exactly the same way as the water alone microcosm experiment, again using pH 5.6 as the basis of comparison. Mean measured total alkalinities ranged from 0.7 mg/l at SAR 5.6 to a low of 0.2 mg/l at SAR 3.5 with no significant differences regardless of SAR treatment level. Measured conductivity change was minor with SAR 5.6 at 83 micromohs compared to 70-81 at other SAR treatment levels. Differences in conductivity were significant comparing SAR 5.6 with SAR 4.5 ($P < 0.05$) but not significant with SAR 4.0 or 3.5. Comparison of pH revealed minor differences in computed means with 5.9 at SAR 5.6 and 5.1-6.3 at other SAR treatment levels. The only level of pH SAR treatment significantly different from SAR 5.6 was 3.5 ($P < 0.05$).

The addition of SAR to microcosms did not result in any clear pattern of impact. Changes in total alkalinity values of the water with sediment present were judged to be too low for accurate measurement with the method used. This is suggested when examining standard deviation (SD) values which were at or near computed means in all except one case. It should also be noted that total alkalinity was essentially at trace levels of detection in all of the microcosms. Conductivity differences were too small to demonstrate any significant differences (excepting SAR 4.5) although all three SAR treatment levels had computed means slightly below that estimated for SAR 5.6. Observations of pH change were also small with significance only at the most acid treatment level (3.5).

Results of this experiment were inconclusive due at least in part to the inability to precisely measure change in total alkalinity and pH with the methods used at the low existing levels of these characteristics. Future evaluations should consider these limitations of measurement by using more sensitive methods.

Conclusions

Over half of 21 ponds and lakes surveyed in Perry and Crawford Counties of extreme south-central Indiana were found to have summer total alkalinity concentrations in the sensitive range for impact from acidic depositions ($n = 11$). A comparison of experimental microcosms with water alone to microcosms with water and sediment revealed sediment in the pond water significantly lowered alkalinity and pH and elevated conductivity. These changes were judged due to edaphic and geologic effects inherent to surface waters in this region of Indiana. Microcosm experiments with water alone treated with SAR at pH 4.5, 4.0, and 3.5 revealed significant impact over that experienced with SAR at pH 5.6, the normal pH for rainwater. Changes observed were an 8-41% lower total alkalinity, a decrease of pH from 0.3-0.4 units, and lowered conductivity from 7-9 umhos. Microcosm experiments with water and sediment had smaller and less conclusive changes than those found for water alone because only trace levels of total alkalinity were found and pH was already at or below about six. Conductivity of the microcosms with water and sediment appeared to be little changed after treatment with SAR. More sensitive methods should be employed to evaluate changes of water quality characteristics such as those found in microcosms with water and sediment.

These results confirm that some surface waters in Perry and Crawford Counties

are sensitive to acidic loading. Future comprehensive field sampling may reveal the exact extent of the potentially sensitive surface waters but the current survey indicates over half may be involved.

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Woody Vegetation of Davis-Purdue Research Forest

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Introduction

The Davis-Purdue Research Forest (DPRF) is a 20.6 ha old-growth deciduous forest dedicated to investigations of long-term forest dynamics. The forest is located in Randolph County (Section 23, T21N, R12E) in east-central Indiana. It is recognized as one of the finest old-growth stands remaining on the Tipton Till Plain and is a Registered National Natural Landmark. The forest was probably grazed before Martha Davis donated the land to Purdue University in 1917 (5). There was limited harvesting of dead and dying trees prior to 1960. In 1971 there was a surface fire of 2-3 hectares in the western part of the forest.

Topography is gently rolling with low relief; elevation changes less than five meters over the forest. Areas of seasonal ponding are scattered throughout the forest. These wetter areas are associated with Pewano and Brookston silty clays. Morley silt loams are found on the well-drained slopes. The somewhat poorly drained Blount silt loam is found on the level higher locations. More extensive descriptions topography and soils can be found elsewhere (5, 6).

Earlier research of DPRF examined compositional dynamics of stems $\geq$ 10 cm dbh (5, 6, 9) or was limited to canopy gaps (6). The objective of this research was to expand the vegetation inventory to include all stems $\geq$ 2 cm dbh (diameter at 1.37 m) and determine which species were present in the 0-1.9 cm dbh size class. This information allows for a more comprehensive examination of stand structure, especially of the large reproduction or sapling size classes. In addition these data will facilitate future research on recruitment in the larger diameter classes by providing baseline data of present conditions.

Methods

In 1986 a 10x10 m grid system was installed in an interior study area (9 ha) of the forest with a transit and 50 m tape. Corners were marked with numbered plastic stakes. The study area was located in the center of the forest to minimize any edge effects on vegetation composition. The data reported here is based on a 100% inventory of the eastern 4 ha (200 x 200m) of the interior study area, which excludes the area burned in 1971.

All woody stems $\geq$ 2 cm dbh were recorded within each 10x10 m quadrat. Stems less than 10 cm dbh were recorded by 2 cm diameter classes: 2.0-3.9 cm, 4.0-5-9 cm, 6.0-7.9 cm, and 8.0-9.9 cm. Stems greater than 10 cm dbh were measured to the nearest 0.1 cm dbh. Five diameter classes were used: small regeneration (2.0-5.9 cm dbh), large regeneration (6.0-9.9 cm dbh), intermediate trees (10.0-19.9 cm dbh), small overstory trees (20.0-49.9 cm dbh), and large overstory ($\geq$ 50 cm dbh). Presence/absence of each species in the 0.0-1.9 cm diameter class within each quadrat was

also recorded. Plants with multiple stems were considered as one individual. For multiple stemmed individuals with the largest stem less than 10 cm dbh, only the largest stem diameter was recorded. The diameters of all stems ≥ 9 cm dbh were recorded when the diameter of the largest stem was greater than 10 cm.

Results

Density

The 4 ha study area contained 5260 stems, ≥ 2.0 dbh, distributed among 40 species (Table 1). An additional six species were present only in the 0.0-1.9 cm diameter class (Table 1). Examination of the diameter strata revealed three distinct vegetation

TABLE 1. Density (stems/ha) by diameter class and species at the Davis-Purdue Research Forest based on a 100% inventory of 4 hectares.

	DIAMETER CLASS[1]					
SPECIES	2.0 – 5.9	6.0 – 9.9	10.0 – 19.9	20.0 – 49.9	>50	TOTAL
Acer negundo	11.0	4.0	2.3	–	–	17.3
Acer rubrum	.3	.5	.3	.3	–	1.3
Acer saccharinum[2]	3.3	1.5	3.3	2.3	.8	11.0
Acer saccharum	412.5	77.0	50.8	17.0	1.3	558.5
Aesculus glabra	41.8	20.5	19.3	2.0	.3	83.8
Asimina triloba	19.5	.3	–	–	–	19.8
Carpinus caroliniana	60.8	20.3	6.8	–	–	87.8
Carya cordiformis	5.3	6.0	4.5	2.5	.3	18.5
Carya glabra	.3	.5	.8	.3	–	1.8
Carya ovata	7.8	9.5	17.3	12.0	.8	47.3
Celtis occidentalis	11.0	2.3	4.5	2.5	–	20.3
Cornus florida	2.3	.5	.3	–	–	3.0
Cornus racemosa	3.3	–	–	–	–	3.3
Corylus americana	1.3	–	–	–	–	1.3
Crataegus sp.	6.0	1.8	5.5	–	–	13.3
Fagus grandifolia	2.0	1.8	5.0	6.8	.3	15.8
Fraxinus americana[3]	8.5	2.5	1.8	6.0	9.0	27.8
Fraximus nigra	.5	.5	1.5	–	.3	2.8
Fraxinus quadrangulata	32.5	7.5	3.3	.5	.3	44.0
Gleditsia triacanthos	–	–	–	1.0	.8	1.8
Juglans nigra	–	–	1.0	7.0	2.0	10.0
Lindera benzoin	17.5	–	–	–	–	17.5
Morus rubra	.8	–	–	–	–	.8
Ostrya virginiana	12.8	3.0	3.3	.3	–	19.3
Platanus occidentalis	–	–	–	.5	.3	.8
Populus deltoides	–	–	–	–	.3	.3
Prunus serotina	3.3	.8	2.0	.8	–	6.8
Quercus alba	–	–	–	1.0	5.3	6.3
Quercus bicolor	2.3	.5	.8	5	2.0	6.0
Quercus macrocarpa	.3	.8	.5	1.8	7.5	10.8
Quercus muehlenbergii	.3	–	–	1.0	3.8	5.0
Quercus palustris	.3	–	–	.5	3.3	4.0
Quercus rubra	–	.5	.3	1.3	11.0	13.0
Quercus shumardii	–	–	–	–	3.0	3.0
Tilia americana	–	.3	–	.5	–	.8
Ulmus americana	31.3	24.8	43.0	17.3	–	116.3
Ulmus rubra	69.3	15.0	16.5	6.5	.3	107.5
Viburnum prunifolium	7.8	–	–	–	–	7.8
Total, stems/ha	775.0	202.3	194.0	91.8	52.3	1315.3

[1]Diameter (cm) at 1.37 m.

[2]Includes individuals of *Acer nigrum*.

[3]Includes individuals of *Fraxinus pennsylvanica*.

complexes: a overstory stratum dominated by mixed oaks, a mesophytic intermediate strata, and the regeneration strata dominated by sugar maple.

Like many of the old-growth forests in the Central Hardwood Region most of the stems in the upper canopy were *Quercus-Carya*, with oak species comprising over 68% of stems greater than 50 cm dbh. *Quercus rubra* was the most common large overstory species on the mesic ridges where it was associated with *Fraxinus americana* and *Quercus alba*. Overstory trees in the seasonally ponded areas formed a *Quercus macrocarpa-Q. palustris-Q. bicolor* complex. Less than 4% of this diameter class was *Acer sp.* or *Ulmus sp.*

While *Quercus sp.* and *F. americana* comprised 80% of the large overstory stems, these species accounted for only 13% of stems in the 20.9-49.9 cm diameter class. The small overstory stratum was not dominated by any one species group. The most abundant species were *Acer saccharum* and *Ulmus americana* with approximately 40% of the stems in this diameter class. Other common species were *Carya ovata* (13% of stems), *Juglans nigra* (8%), *Fagus grandifolia* (7%), and *Ulmus rubra* (7%).
(7%).

Acer saccharum, U. americana, and *U. rubra* were the dominant species in the 10.0-19.9 cm diameter class with 26%, 22%, and 9% of all stems, respectively. The hickories—*Carya ovata, C. cordiformis*, and *C. glabra*—accounted for another 12% of stems. Slightly less than 10% of stems were *Aesculus glabra*. Only six oaks were found in this diameter class in the 4 ha study area.

The trend of increasing dominance by *Acer saccharum* into smaller diameter classes continued with the large regeneration. Nearly 40% of stems 6.0-9.9 cm dbh were *A. saccharum*. *Ulmus americana*, *A. glabra*, and *U. rubra* accounted for 12%, 10%, and 8% of stems, respectively. Understory species[1], species which do not grow large enough to form part of the canopy, were of increased importance, 12% of stems, in this diameter class compared to the larger diameter classes. Slightly over 10% of the large regeneration was *Carpinus caroliniana*.

There were over 400 stems/ha of *A. saccharum* in the small regeneration diameter class. Although *Ulmus sp.* comprised less than 13% of the small regeneration stems, their combined density was still over 100 stems/ha. Two other species had relatively high small regeneration densities: *A. glabra* (61 stems/ha) and *Fraxinus quadrangulata* (32 stems/ha). Together the understory species accounted for over 16%, 125 stems/ha, of all small regeneration stems. Of the 19 oaks $\leq$ 10.0 cm dbh found in the study area, 11 were *Q. bicolor*.

Frequency

A modified perspective of woody vegetation structure is seen by examining frequency of occurrence, the proportion of the 400 quadrats occupied by the different species in each diameter class (Table 2). The high relative density of *A. saccharum* in the regeneration diameter classes, 53 and 38% of all stems, does not necessarily translate to its eventual domination of the overstory. While small regeneration of *A. saccharum* was found in four-fifths of the quadrats; less than one-fifth of the quadrats had at least one *A. saccharum* larger than 20 cm dbh. Intermediate stems of *A. saccharum* were absent in 60% of the quadrats having an intermediate sized stem.

A number of species were far more frequent than would be suggested by their relative densities. *Fraxinus americana* and *C. cordiformis* each accounted for less than 1% of the large regeneration, yet were found in 12% and 10% of the quadrats respectively. Large regeneration of *F. quadrangulata* grew in one-fifth of the quadrats. The elms were widely dispersed, both species were observed in over two-thirds of the quadrats.

TABLE 2. Frequency (% of 10 × 10m quadrats occupied) by diameter class and species at the Davis-Purdue Research Forest based on a 100% inventory of 4 hectares.

	DIAMETER CLASS[1]						
SPECIES	0.0 −1.9	2.0 −5.9	6.0 −9.9	10.0 −19.9	20.0 −49.9	>50	TOTAL
Acer negundo	7.5	9.0	3.5	2.0	–	–	17.3
Acer rubrum	1.5	.3	.5	.3	.3	–	2.0
Acer saccharinum[2]	3.3	3.0	1.5	2.5	1.3	.8	7.8
Acer saccharum	79.9	78.3	56.5	32.8	15.0	1.3	95.8
Aesculus glabra	52.3	25.3	17.5	16.5	2.0	.3	73.0
Asimina triloba	9.8	6.8	.3	–	–	–	10.3
Carpinus caroliniana	20.0	28.0	17.8	6.5	–	–	50.0
Carya cordiformis	10.8	4.8	5.3	4.5	2.3	.3	23.5
Carya glabra	–	.3	.5	.8	.3	–	1.5
Carya ovata	5.0	7.0	8.5	12.5	11.8	.8	33.3
Celtis occidentalis	12.5	9.0	2.3	4.0	2.5	–	25.0
Cephalanthus occidentalis	.3	–	–	–	–	–	.3
Cercis canadensis	.3	–	–	–	–	–	.3
Cornus florida	2.5	2.0	.5	.3	–	–	4.5
Cornus racemosa	6.3	2.3	–	–	–	–	7.0
Corylus americana	.8	.5	–	–	–	–	.8
Crataegus sp.	17.8	5.0	1.8	4.3	–	–	25.5
Euonymus sp.	4.0	–	–	–	–	–	4.0
Fagus grandifolia	1.5	2.0	1.8	4.5	6.8	.3	15.8
Fraxinus americana[3]	11.8	6.5	2.3	1.8	5.5	9.0	29.5
Fraxinus nigra	.5	.5	.5	1.0	–	.3	2.5
Fraxinus quadrangulata	21.3	18.5	6.5	3.3	.5	.3	34.3
Gleditsia triacanthos	–	–	–	–	1.0	.8	1.8
Juglans nigra	–	–	–	1.0	6.5	2.0	9.5
Lindera benzoin	38.8	9.5	–	–	–	–	39.5
Morus rubra	1.8	.8	–	–	–	–	2.5
Ostrya virginiana	7.0	7.5	2.5	2.8	.3	–	13.3
Platanus occidentalis	–	–	–	–	.5	.3	.8
Populus deltoides	–	–	–	–	–	.3	.3
Prunus serotina	3.3	3.3	.8	1.8	.8	–	9.8
Quercus alba	.3	–	–	–	1.0	5.3	6.3
Quercus bicolor	1.8	2.0	.5	.8	.5	2.0	6.8
Quercus macrocarpa	1.0	.3	.8	.5	1.8	6.8	10.3
Quercus muehlenbergii	–	.3	–	–	1.0	3.8	5.0
Quercus palustris	.5	.3	–	–	.5	3.3	4.3
Quercus rubra	.8	–	.5	.3	1.3	10.0	12.8
Quercus shumardii	–	–	–	–	–	2.8	2.8
Sambucus canadensis	5.3	–	–	–	–	–	5.3
Staphylea trifolia	1.0	–	–	–	–	–	1.0
Tilia americana	–	–	.3	–	.5	–	.8
Ulmus americana	21.3	23.3	21.5	32.3	15.8	–	69.3
Ulmus rubra	40.8	34.8	14.5	13.5	6.0	.3	66.0
Viburnum dentatum	.5	–	–	–	–	–	.5
Viburnum prunifolium	21.8	5.0	–	–	–	–	23.5
All species combined	99.0	99.8	85.3	85.0	60.5	44.8	100.0

[1]Diameter (cm) at 1.37 m.

[2]Includes individuals of *Acer nigrum*.

[3]Includes individuals of *Fraxinus pennsylvanica*.

Discussion

The results of this research are consistent with other research in oak-hickory forests which found increased importance of late seral species in the smaller diameter classes (1, 3, 4, 6). *A. saccharum* was both the most numerous and frequent species

in the regeneration strata. However, the wide dispersion of the regeneration of many species allows future stand development to proceed along several alternative routes depending on future disturbance patterns.

Eventual conversion of the upper canopy to an *A. saccharum* association must be qualified. Many of the small maples in the seasonally ponded areas were established on micro-hammocks (<4m^1). The small size of these hammocks limits the development of flood sensitive species such as *A. saccharum* (8). As the trees on the hammocks grow into the larger diameter classes there is insufficient root space in mesic soil to support the trees during periods of high water tables. The summer of 1986 had higher than average rainfall and we observed many intermediate-sized maples on micro-hammocks exhibiting symptoms of fooding stress. Another qualification is the decreasing relative importance of *A. saccharum* in the larger diameter classes noted in the results section. While over 50% of small regeneration was *A. saccharum*; only one-quarter of the intermediate diameter class were maple.

The low stand regeneration densities exhibited by *U. rubra, C. ovata* and *F. grandifolia* appears to be related to a factor which limits their establishment, rather than a factor which limits their development. The density of these species declined only slightly or was stable with increasing diameter class.

One and two year old oak seedlings were common throughout the forest (pers. obs.). However, growth of oak seedlings appears to be restricted to the areas of seasonal ponding where many of the more shade tolerant, and flood intolerant, species were excluded.

Without a change in the disturbance regime the densities of many of the mid-successional species will most likely continue to decline and some early successional species will eventually be lost from the stand. Six species with stems in the overstory strata did not have stems in the regeneration diameter class; and the regeneration density of seven other species was $\leq$ 1 stem/ha. The aesthetic appeal of managing forests as preserves, allowing nature to run its course, must be balanced by the consequence of a possible decline in biotic diversity.

Acknowledgments

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Note

[1]*Asimina triloba, Carpinus caroliniana, Cornus sp., Corylus americana, Lindera benzoin, Ostrya virginiana*, and *Vibrunum prunifolium*.

ENGINEERING

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ABSTRACTS

Bioelectromagnetic Absorption Measurement Methods. DAVID D. CHESAK, Saint Joseph's College, Rensselaer, Indiana 47978.——The absorption of non-ionizing electromagnetic radiation by humans continues to be of concern as the sources of radiation proliferate in both the civilian and military sectors. This presentation deals with measuring techniques for determining the effect of this radiation within simulated human subjects. The instrumentation for this is discussed.

Application of Low Rate Speech Coding Techniques to the Production of Synthetic Pipe Organ Sounds. JOEL R. CROSMER, Valparaiso University, Valparaiso, Indiana 46383.——A method of generating pipe organ-like sounds with techniques used in low rate speech coding is described in this paper. In this method, the similarities between an organ pipe and the human vocal tract are exploited to produce more natural sounding musical notes. The author demonstrates that the filter/excitation sound production model is useful for giving each note in a keyboard scale unique characteristics. This is accomplished by modifying its steady state spectral characteristics, as well as the attack and decay properties, through changes in time varying synthesis filters. It is also shown that subtle phase variations in acoustically generated sounds can be introduced by small perturbations of the filter parameters.

Microcomputer Course for All Engineering Students. RICHARD L. GONZALES, Department of Engineering, Purdue University, Calumet, Hammond, Indiana 46323.——A new sophomore lecture and laboratory course in microcomputer and microcontroller applications for all engineering students is being developed and implemented at Purdue University Calumet. The equipment required is partially supported by an NSF CSIP grant. The course contains a significant laboratory component and uses software and hardware that is uniquely suited to the project. The outstanding feature of the course is the plan to take students, without prior knowledge of the subject, to a point where they can produce, in the laboratory, a turn key microcontroller system. This is considered the minimal level of knowledge necessary for all engineering students.

A Scientist's Perspective on the Strategic Defense Initiative. WILLIAM A. HOLLERMAN, Nichols Research Corporation, 4040 S. Memorial Parkway, Huntsville, Alabama 35802.——Since President Reagan created the Strategic Defense Initiative (SDI) in 1983, political issues have played a large role in the development of a defense system which would shield the United States from the threat of nuclear armageddon. For the average scientist working in the SDI arena, political issues do not enter into the day-to-day development of particular shield elements. This report will present an overview of current SDI concepts and technologies from a scientist's perspective. A careful attempt will be made to avoid *all* political issues.

Use of Bottom Ashes in Indiana Highway Construction. Wei-Hsing Huang and Charles W. Lovell, School of Civil Engineering, Purdue University, West Lafayette, Indiana 47907.——Indiana coal-fired power plants produce large quantities of bottom ash. These materials are often wasted, but are potentially useable in highway construction for embankment, subgrade and subbase material. In order to use them in this manner, they must be experimentally characterized both physically and chemically. A total of 11 bottom ashes were collected from all parts of the state and tested for (1) grain size distribution, (2) specific gravity, (3) complete chemical analysis, and (4) X-ray diffraction. This paper presents the results of laboratory studies determining the physical and chemical properties of the material.

ENTOMOLOGY

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ABSTRACTS

Wheat Growth as Affected by Barley Yellow Dwarf Virus. Jaime E. Araya, Alberto Fereres and John E. Foster, Department of Entomology, Purdue University, West Lafayette, Indiana 47907.——An experiment with hydroponic cultures was designed to study the effects of infection with barley yellow dwarf virus (PAV isolate) on growth of roots and foliage of wheat plants. The seedlings were infested at the 1-2 leaf stage with viruliferous bird cherry-oat aphids, *Rhopalosiphum padi* (L.). The aphids were eliminated 48h after infestation with pyrethrum aerosol. Virus infection delayed the growth of roots and leaves, as compared with non-infected control plants, with small differences among wheat cultivars.

Allozyme Charaterization in the *Aedes (Stegomyia) albopictus* Sub-Group. David Colón-Arroyo and Karamjit S. Rai, University of Notre Dame, Notre Dame, Indiana 46556.——Since its official discovery in the fall of 1985 in Houston, *Aedes albopictus* has received a fair amount of attention due to its potential as a vector for Dengue Fever and Dengue Hemorrhagic Fever, two debilitating and sometimes fatal diseases common in Southeast Asia and the Caribbean belt. Here we analyze allozyme mobility in four species in the *albopictus* sub-group and two hybrid combinations. As expected in lab colonies there is a low degree of heterozygosity as compared to field populations. Further morphometric measurements suggest hybrid vigor in the fertile offsprings. If size is directly related to vector competence, these hybrids could present an additional hazard regarding the transmission of these diseases.

Probing and Feeding Behavior of the English Grain Aphid, *Sitobion avenae* (F.), on BYDV-infected Wheat. A. Fereres, R. H. Shukle, J. E. Araya and J. E. Foster. Department of Entomology, Purdue University, West Lafayette, Indiana 47907 and USDA-ARS.——An electronic device was used to study feeding of *S. avenae* on wheat infected with the PAV and RPV isolates of barley yellow dwarf virus (BYDV). The system applies an electric current through an aphid attached to a thin gold wire. The aphid serves as a variable impedance and the resulting signal modulations are amplified and recorded on strip charts as waveforms from each particular aphid feeding event.

Aphids feeding on BYDV-infected wheat plants had no significant differences in feeding behavior when compared with non-infected plants, except for some differences ($P<0.10$) in the number of phloem contacts. When the cultivars were infected with PAV, aphids fed better on 'Abe' (susceptible) than on 'Caldwell' (tolerant), as indicated by less probes, a shorter salivation time, less time for a committed (longer

than 30 min) phloem ingestion, and less phloem contacts on the BYDV-susceptible cultivar than on the tolerant one. No cultivar differences were found when aphids fed on healthy plants.

Geographical Distribution and Vertebrate Host Records for Ticks Submitted to Rocky Mountain Spotted Fever Tick Testing Program, 1982-1986. TODD GLANCY AND ROBERT R. PINGER, Department of Physiology and Health Science, Ball State University, Muncie Indiana 47306.——Geographical distribution and vertebrate host records are an important by-product of the Rocky Mountain Spotted Fever Tick Testing Program. The Program, which began in 1982, has been funded in part by the Indiana State Board of Health from 1983-present. The Public Health Entomology Laboratory maintains records on each tick submitted. For the period 1982-1986, there are records on 11,657 ticks. Ten species of ticks have been identified, including three species which were probably imported from out of state, *Amblyomma maculatum, Ixodes dammini*, and *I. scapularis. Dermacentor variabilis* ticks were received from every county but Benton County. Specimens of *A. americanum* which normally is limited to the southern part of the state were received from as far north as Allen, Howard, and St. Joseph Counties.

Container Breeding Mosquitoes of East Central Indiana. WENDI L. GRAY, Box 182, Parker, Indiana 46368, and ROBERT R. PINGER, Department of Physiology and Health Science, Ball State University, Muncie, Indiana 47306.——Since the last comprehensive study of mosquitoes in east central Indiana was completed, two potential vectors have appeared in Indiana. Both of these species, *Aedes albopictus* and *Aedes atropalpus*, are known as container breeders. LaCrosse virus (California encephalitis virus group) has been isolated from a third species of container breeding mosquitoes, *Aedes triseriatus*, collected in Delaware County. Each of these species colonizes used automobile and truck tires and it is probable that the two former species entered Indiana by way of transported tires. To determine the presence or absence of *Aedes albopictus* and *Aedes atropalpus*, adult and larval mosquitoes were collected from six east central Indiana tire and retreading services from June 6 to September 30, 1987. Each collection site was characterized by pH, temperature, and the amount of sun or shade present. Samples of *Aedes triseriatus* were sent to the University of Notre Dame for virus isolation attempts and a checklist of container breeding mosquitoes from the area was compiled.

A Key to the Alate Forms of Genera of Indiana Ants (Formicidae: Hymenoptera) JACK R. MUNSEE, Department of Life Sciences, Indiana State University, Terre Haute, Indiana 47809.——Because most keys for ant identification are based on worker castes, identification of alate forms, males and queens, is not easy unless such forms are collected simultaneously with workers of the same species. Also, male and female or queen ants of the same species usually are greatly differentiated, especially as to size, ocelli, and antennal characteristics. Wings of alate forms, of the same genera, however, are more similar as to shape and pattern of wing venation.

The attempt is made to use the patterns of wing venation and the cells formed by the veins to construct a dichotomous key to separate the genera of the ants of Indiana. Such a key would be especially useful for determining the genus where the basis for determination depends upon wings only as might be the case with queen ants since they lose their wings soon after mating. Male ants are apt to retain their wings.

Different Sorghum Genotypes Evaluated for Resistance to the Maize Weevil, *Sitophilus zeamais*, (Motschulsky), (Coleoptera: Curculionidae). MUSTAPHA EBRIMA NJIE. Purdue University, West Lafayette, Indiana 47907.——A total of 118 different sorghum genotypes were screened for resistance to the maize weevil using both "free and no-choice" design commonly called the "cafeteria". All but one of the experiments were done under conditions of optimum temperature, relative humidity (R.H.). and grain moisture content. The exception was done under low humidity conditions (i.e. 45% R.H.). Some of the tests were studied for 70 days, and some for a longer period of 100 days. Criteria used for evaluation were preference, progeny produced, weight loss of grain and developmental period of the larva. Individual lines were evaluated for tannin content, endosperm hardness, and density. None of these factors seemed to have had an effect. However, in the 100-day tests, it was found that the weevils died rather than consume pericarp. Hence, pericarp is probably a factor for resistance to the maize weevil in sorghum.

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New Distribution Records for *Aedes atropalpus* Breeding in Tires in Indiana

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The Rockhole Mosquito, *Aedes atropalpus* (Coquillett), was first discovered in Indiana breeding in discarded tires at a St. Joseph County auto salvage yard in 1979 (Restifo and Lanzaro, 1980). Previously, *Ae. atropalpus* was not reported from Indiana, Ohio, or Kentucky, though it was found in Michigan and might be found in Indiana as well (Siverly, 1972). Subsequently, several more populations of *Ae. atropalpus* have been found in Indiana.

During the summers of 1986-87, the Vector Control Section of the Indiana State Board of Health conducted an extensive survey of tire accumulations for *Aedes albopictus*. In the course of this statewide survey, approximately 200 tire dealerships, retreaders, and scrap tire piles were examined and recorded. Tire sites believed to be at higher risk of infestation were inspected several times during each season. In addition to the discovery of three *Ae. albopictus* populations, nine infestations of *Ae. atropalpus* were recorded. This information is of interest because *Ae. atropalpus* was previously not known to be indigenous to Indiana, and its presence is likely to reflect interstate movement of tires. Hence, *Ae. atropalpus* may serve as an indicator for sites at risk of infestation by *Ae. albopictus*.

The following are records of adult and larval collections of *Ae. atropalpus* from tire sites in Indiana.

Marion County, Indianapolis, Indiana:

One larva was collected at a salvage yard by a technician from Marion County mosquito control on September 1, 1984. A subsequent survey of the salvage yard on September 10 yielded 12 larvae from a flooded auto trunk. The larvae are now commonly found in tires and flooded auto bodies throughout the site. The *Ae. atropalpus* larvae are found often in association with *Culex pipiens* (Linnaeus), *Culex restuans* Theobald, and, occasionally *Aedes triseriatus* (Say). Chironomids are sometimes found in flooded trunks and cars as well, particularly if a flocculent sediment is present. Containers in which *Ae. atropalpus* larvae were found often contain such sediment or a sparse algal growth.

Marion County, Indianapolis, Indiana:

On September 16, 1987, three dead larvae were collected from a truck tire behind a retreading shop. The larvae were found during a post-treatment survey of the site for *Ae. albopictus*. Though *Ae. albopictus* remains at this site, no *Ae. atropalpus* larvae or adults have been found since. This retreading shop is approximately eight miles from the other *Ae. atropalpus* site.

Knox County, Wheatland, Indiana:

One fourth-instar larva and three pupae (allowed to emerge as adults in the laboratory) were collected from a single truck tire on September 17, 1986. This tire was one of several hundred located behind a retreading and repair shop. Many of these tires contained clear water with algae or a silty sediment.

Morgan County, Martinsville, Indiana:

Six *Ae. atropalpus* larvae were collected on July 1, 1987, from a pile of about 10,000 scrap tires dumped on a bulldozed hilltop. These tires had been in place several months; since they contained no leaf litter they were probably dumped the previous winter. The tires had not yet been extensively colonized by mosquitoes of any species. The few larvae that were collected were found in association with large numbers of small "tadpoles." Water in the tires was generally clear with silty sediment or algal growth on the bottom. No adults were collected at this time. Another inspection on September 30, 1987, yielded no *Ae. atropalpus* larvae, but landing-biting collections revealed *Ae. atropalpus* to be the predominant biter in the vicinity of the tire pile. In one such collection, 15 *Ae. atropalpus* and 5 *Ae. triseriatus* were observed biting in a 20-minute period.

Kosciusko County, Atwood, Indiana:

Adults and larvae were collected from tires on the perimeter of an old gravel pit filled with approximately six million tires on August 25, 1987. Other mosquito larvae collected included *Culex pipiens, Cx. restuans, Cx territans, Walker, Anopheles punctipennis* (Say, and *Ae. triseriatus.*

Lake County, Lake Station, Indiana:

On September 25, 1987, five female *Ae. atropalpus* were collected at a retread outlet specializing in large-implement tires. This site is in an urban area in a fenced lot with little vegetation. Other mosquitoes collected included *Cx pipiens, Cx territans*, and *An. punctipennis.*

Warrick County, Boonville, Indiana:

A resting collection of 10 female and 14 male *Ae. atropalpus* was made from large-implement tires outside of a tire dealership on September 25, 1987. Two larvae and one pupa were also collected. A single biting female was captured as well.

Dubois County, Jasper, Indiana:

An adult female *Ae. atropalpus* was collected on September 28, 1987 at a tire dealership where farm-implement tires were vertically stacked and stored outdoors. One adult male was also collected from flowering plants adjacent to the tires.

Harrison County, Corydon, Indiana:

One adult female *Ae. atropalpus* was collected on September 30, 1987 in a wooded area west of a two-acre pile of scrap tires. Eighty-five *Ae. triseriatus* and one *Aedes vexans* (Meigen) were also collected. Resting collections of adult mosquitoes from inside the tires yielded eight female *Ae. atropalpus*, two male *Ae. atropalpus*, and two *Ae. triseriatus.*

Vanderburgh County, Evansville, Indiana:

A resting collection from truck and implement tires at a tire dealership yielded four female and seven female *Ae. atropalpus* on October 2, 1987.

Acknowledgment

The authors wish to thank Dr. Michael J. Sinsko (ISBH) for reviewing this manuscript, Barbara Blue (MCHD) for reporting the collection of the initial Marion County specimen, and Dr. George B. Craig, for urging publication of these data.

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Distribution, Checklist and Key to Adult Tiger Beetles (Coleoptera: Cicindelidae) of Indiana

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Introduction

Early work on Indiana tiger beetles by Blatchley (1), Goldsmith (5), and Montgomery and Montgomery (7) gave information on the distribution of species in the State. Shelford (11, 12, 13) contributed much to the understanding of tiger beetle natural history from his studies of species of the Lake Michigan Dunes area. Recently, Knisley (6) surveyed tiger beetles from the dunes region and Munsee (8) and Schrock (9) sampled tiger beetles on unreclaimed coal stripmine spoil banks in western Indiana. Our intent with this paper is to provide a current review of this popular and widely collected group of insects in Indiana so that changes in distribution and abundance through habitat degradation or other factors might be studied. Results of this study are based on our recent collecting throughout the State and an examination of museum collection records. A checklist and taxonomic key for the species we think are present in the State are provided. The keys of Blatchley (1) and Willis (14) include different species and are not suitable.

Methods

Most museum and private collections expected to have a good representation of Indiana tiger beetles (see checklist legend) were examined. Museum specimens typically had older dates and indicated a decline in recent collecting. Most of the recent records were obtained from personal collections of Downie, Brzoska, and Knisley, the Munsee and Schrock surveys (8, 9, 10), and unpublished work of Knisley. Determinations of seasonal pattern of occurrence and habitat preference were based on our observations, collection records, and published information of these species.

Results and Discussion

Blatchley (1) lists 16 species and an additional variety of Cicindelidae from Indiana. He also speculated that *C. marginipennis* "very probably occurs along the streams of the southeastern portion of Indiana." Montgomery and Montgomery (7) added *C. celeripes* to the state list, although we consider this species to be *C. cursitans*, a morphologically similar species. *Cicindela celeripes* is common further west and probably does not occur in Indiana. The checklist of North and Central American species by Boyd (3) is a literature compilation of published records which includes for Indiana the species on Blatchley's list plus *C. limbalis* and *C. splendida*. Our checklist of 20 species includes several species name changes (following Boyd et al. 1982), the addition of three species (*C. marginipennis, C. splendida, C. cursitans*), and the deletion of *C. ancocisconensis*. The name changes are: *C. cuprascens macra*

to *C. macra, C. purpurea limbalis* to *C. limbalis, C. vulgaris* to *C. tranquebarica, C. generosa* to *C. formosa generosa*, and *C. lecontei* to *C. scutellaris lecontei.*

We have collected all of the species on our checklist except *C. splendida* and *C. limbalis*. These two species are taxonomically similar and may be the same species (Walter Johnson, personal communication). *Cicindela splendida* has been collected in southern Ohio (4) and we found suitable habitat in southern Indiana. *Cicindela limbalis* has been collected in northern Ohio (4) and near Chicago, but no suitable habitat was found during limited surveys in nothern Indiana. We collected *C. cursitans* at one site in Posey County and *C. marginipennis* in three southeastern counties. We did not include *C. ancocisconensis* on our list because we did not find it during our collecting or in any of the collections which we examined. Blatchley (1) reported it from Fulton County along Bruce Lake on July 14, an unlikely habitat and date. Goldsmith (5) reported it from water edge sites in several counties and made some observations on it along the Jordan River in Bloomington. Since this species is easily confused with *C. repanda*, typically occurs along mountain streams, and is more eastern in distribution, these records are dubious.

The three types of life history patterns recognized by Shelford (12) as typical of tiger beetles are represented among the Indiana species. Adults of summer species appear in June and mate, oviposit, and die off by early fall. Fall-spring adults emerge about late August, feed for about six weeks, overwinter, and then re-emerged in spring to mate and reproduce. *Cicindela sexguttata* is the only spring species in Indiana. Adults appear in April and usually disappear by late June. We found very few individuals in late summer indicating little or no fall emergence as may occur for this species in the southeastern U.S. (13). The life history of *C. hirticollis*, a fall-spring species, is quite interesting because adults are present from late spring through fall. Shelford (12) indicates that this is because the new adults emerge about mid-July while overwintered adults from the previous brood are still present.

Examination of museum collections was made possible with the kind help of: Dr. Wallace LaBerge, Illinois Natural History Survey; Dr. George Byers, Snow Entomological Museum; Dr. W.P. McCafferty and A. Provonsha, Purdue University; Dr. Frank Young, Indiana University-Bloomington; and Dr. John Dustman, Indiana University-Gary. Dr. Gary Dunn provided records from the University of Michigan, Florida Division of Plant Industry, and other museum records. Knisley obtained records from collections, collected throughout the State, and revised much of the text. Brzoska confirmed many identifications, provided life history information and adapted the key from his work with Ohio tiger beetles (4). Schrock visited museums, solicited specimens, wrote the draft text and prepared range maps.

CHECKLIST OF ADULT TIGER BEETLES KNOWN FROM INDIANA

BK = Barry Knisley collection
DB = David Brzoska collection
GD = Records from Gary Dunn
IL = Illinois Natural History Survey
ND = N.M. Downie collection
ISU = Indiana State University
IUG = Indiana University-Gary
KU = Snow Entomological Museum, University of Kansas
PU = Purdue University
B = Blatchley (1)

G = Goldsmith (5)
K = Knisley (6)
M = Montgomery and Montgomery (7). Also includes records from Purdue Univ. and from personal collections of V.E. Shelford and A.B. Wolcott.
S = Munsee and Schrock (8, 9, 10)

1. *Megacephala virginica* (Linnaeus) B, M, GD, ND, ISU, PU
(= *Tetracha virginica*)
Summer species, found between June and September; habitat generalist; often found in fields; nocturnal and comes to lights where it feeds on insects; not clear if it is attracted by light or by the insects.
2. *Cicindela cuprascens* LeConte B, M, BK, DB, GD, ND, IL, IUG, PU
Summer species; known mainly from sand bars along rivers.
3. *Cicindela cursitans* LeConte M, BK, PU
Summer species; known only from southwest corner of state; runs rapidly, flightless; damp areas back from water edge.
4. *Cincindela duodecimguttala* Dejean B, M, BK, DB, GD, ISU, IUG, PU
Fall-spring species; found along the banks of creeks.
5. *Cicindela formosa generosa* Dejean B, G, K, M, BK, DB, ND, IL, ISU, PU
(= *C. generosa*)
Spring and fall populations; occurs in deep sand areas such as sand quarries.
6. *Cicindela hirticollis* Say B, K, M, BK, DB, ND, IL, PU
Fall-spring species but overlap results in presence during summer; common on wet areas in sand dunes, beaches, sand bars and rivers.
7. *Cicindela lepida* Dejean B, K, BK, DB, GD, ND, IUG, PU
Summer species; found only on fine white sand, mostly in Dunes area; can be collected at night with a light.
8. *Cicindela limbalis* Klug
(= *C. purpurea limbalis*)
Fall-spring species; may occur along northern border of state (occurs in IL, OH); habitat on vertical clay banks, roadcuts, washouts.
9. *Cicindela macra* LeConte K, BK, DB, ND, PU
(= *C. cuprascens macra* of Blatchley)
Summer species; abundant along Lake Michigan beaches.
10. *Cicidela marginipennis* Dejean BK, DB
Summer species; found on gravel bars on rivers; found only in southeast corner of State.
11. *Cicindela patruela* Dejean B, M, BK, DB, IL, IUG, KU, PU
Fall-spring species; paths in sandy forests.
12. *Cicindela punctulata* Olivier B, G, K, M, BK, DB, ND, ISU, PU
Summer species; common in wide range of habitats; found on barren acidic spoilbanks, attracted to lights.
13. *Cicindela purpurea* Olivier B, G, M, BK, PU
Fall-spring species; probably more widespread than indicated; occurs quite early in season (March and April).
14. *Cicindela repanda* Dejean B, G, K, M, BK, DB, ND, IL, ISU, IUG, PU
Fall-spring species; generalist but requires some moisture.
15. *Cicindela rufiventris* Dejean B, K, M, DB, GD, ND, ISU, IUG, PU
Summer species; prefers gravel areas, roadcuts, bare slopes.

16. *Cicindela sexguttata* Fabricius B, G, K, M, S, BK, DB, ND, IL, ISU, IUG, KU, PU
Spring species with little or no fall emergence; generally found in open woodlands; captured on stripmines in grassy and young wooded plots.
17. *Cicindela scutellaris lecontei* Haldemann B, K, M, BK, DB, ND, IL, ISU, PU
Fall-spring species; most frequently found on sparsely vegetated sandy areas, usually with *C. formosa generosa*.
18. *Cicindela splendida* Hentz
Fall-spring species; should be found in southern half of state (occurs in IL, OH); found on clay banks and open areas.
19. *Cicindela tranquebarica* Herbst B, G, M, BK, DB, ND, IL, ISU, IUG, PU (= *C. vulgaris*)
Fall-spring species; generalist; sandy or clay areas.
20. *Cicindela unipunctata* Fabricius M, S, BK, GD, ND, IUG, KU, PU
Summer species; adult population peaks in June; crepuscular/nocturnal; moved onto newly wooded spoilbanks; weak flyer.

KEY TO SPECIES OF ADULT TIGER BEETLES OF INDIANA

(adapted from Brzoska (4) and Willis (14)

1. Anterior angles of pronotum more prominent than anterior margins of prosternum (Fig. 2B); third segment of maxillary palps longer than the fourth (Fig. 3B) ..... *Megacephala virginica* (Linnaeus)
1′. Anterior pronotal angles not more prominent than prosternal margin (Fig. 2A); third segment of maxillary palps shorter than fourth (Fig. 3A) ..... genus *Cicindela* 2
2(1′) Front and middle trochanter without subapical setae (Fig. 4B); venter of abdomen reddish brown of testaceous 3
2′. Front and middle trochanter with one or two subapical setae (Fig. 4A); venter of abdomen metallic green or blue 4
3(2). Markings of elytra connected at margin; elytra not microserrulate; elytra dull green to olive brown ..... *C. marginipennis* Dejean
3′. Markings of elytra not connected at margin (Fig. 14); elytra microserrulate; elytra dull black ..... *C. rufiventris* Dejean
4(2′) Clypeus densely to sparsely clothed with decumbent setae (Fig. 5B) ... 5
4′. Clypeus glabrous or with a few erect setae (Fig. 5A) 7
5(4). Appendages without pigmentation (pale tan) ..... *C. lepida* Dejean
5′. Appendages pigmented 6
6(5)′ Elytra dull, shallowly punctate; female elytral apices acute ..... *C. macra* LeConte
6′. Elytra shiny, deeply punctate; female elytral apices rounded ..... *C. cuprascens* LeConte
7(4′) Frons with supraorbital setae only (middle portion of frons glabrous) (Fig. 6A) 8
7′. Frons and/or vertex with setae (in addition to supraorbitals) (Fig 6B) 14
8(7). Small, less than 9mm in length 9
8′. Large, more than 9 mm in length 10
9(8). Elytra notably expanded in apical one-half, marking usually reduced, without complete marginal line; legs metallic green (this species probably does not occur in Indiana but is very similar to *C. cursitans*) ..... *C. celeripes* LeConte

9′. Elytra not notably expanded apically; usually with marginal line, legs light brown, only slightly metallic ..... *C. cursitans* LeConte
10(8′) Proepisternum with setae (only a few near coxal margin) (Fig. 7B) .. 11
10′. Proepisternum glabrous (Fig. 7A) ..... *C. unipunctata* Fabricius
11(10). 1st antennal segment with one sensory seta (Fig. 8A) ..... *C. punctulata* Olivier
11′. 1st antennal segment with three or more sensory setae (Fig. 8B) ... 12
12(11). 1st antennal segment with more than four sensory setae (Fig. 12) ..... *C. scutellaris lecontei* Haldemann
12′. 1st antennal segment with three or four sensory setae 13
13(12′) Elytra granulate; lateral margins of abdomen with sparse decumbent setae; middle band usually complete .... *C. patruela* Dejean
13′. Elytra shallowly to deeply punctuate; abdomen glabrous laterally; middle band usually broken into dots or absent ..... *C. sexguttata* Dejean
14(7′). Genae with setae (Fig. 9B) 15
14′. Genae glabrous (Fig. 9A) 22
15(14). Labrum unidentate (Fig. 10A), or nondentate 16
15′. Labrum tridentate (Fig. 10B), or with more teeth 17
16(15). Pronotum narrow (proportions: 4 units long, 5 wide), front angles rounded (Fig. 11A); humeral lunules usually complete and connected to or slightly separated from marginal line (Fig. 11C) ..... *C. repanda* Dejean
16′. Pronotum wide (proportions: 4 units long, 6 wide), front angles acute (Fig. 11B); humeral lunules usually broken and/or widely separated from marginal line (Figs. 11D, 15) ..... *C. duodecimguttata* Dejean
17(15′) Elytral markings broad and connected by complete marginal band (Fig. 16) ..... *C. formosa generosa* Dejean
17′. Elytral markings not connected by complete marginal band 18
18(17′) Humeral lunule long and oblique, projecting strongly mesad, may or may not be connected to the marginal markings (Figs. 13, 17) .... *C. tranquebarica* Herbst
18′. Humeral lunule absent or short 19
19(18′) Marginal line extending nearly to apical lunule, all lunules complete (Fig. 18) ..... *C. ancocisconensis* Harris
19′. Marginal line poorly developed or absent 20
20(19′) Head and pronotum much differently colored from elytra, head and thorax usually green or blue, elytra reddish (Fig. 19) ..... *C. splendida* Hentz
20′. Head, pronotum and elytra about the same color (may have contrasting margins), reddish or rarely greenish 21
21(20′) Humeral lunule absent, middle band narrow, nearly straight (Fig. 20) ..... *C. purpurea* Olivier
21′. Humeral lunule present, middle band wider, more sinuate (Fig. 22) ... . *C. limbalis* Klug
22(14′) Humeral lunule long and oblique, projecting strongly mesad, may or may not be connected to the marginal markings (Fig. 17) .... *C. tranquebarica* Herbst
22′. Humeral lunule not long and oblique 23
23(21′) Elytral markings complete; middle band sinuate and extending nearly to midline (Fig. 21) ..... *C. hirticollis* Say
23′. Elytral markings incomplete and variable, middle band represented as a dot or triangular marking along margin reaching less than half way to midline (Fig. 23) ..... *C. scutellaris lecontei* Haldemann

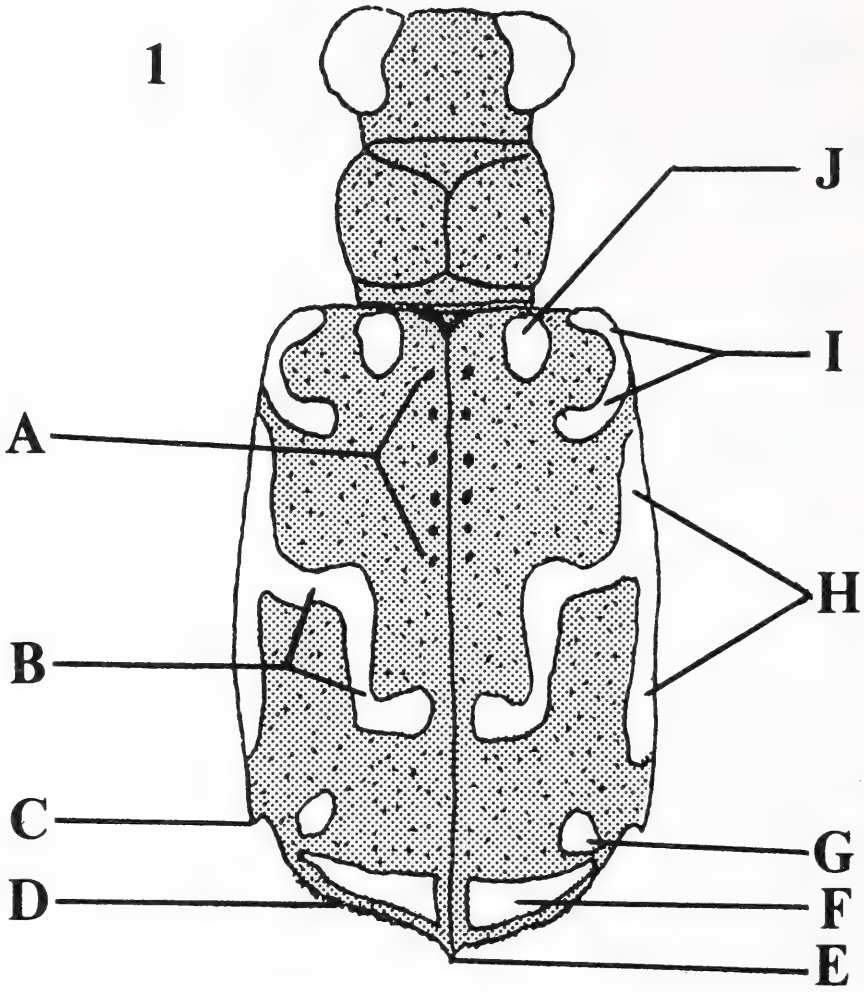


FIGURE 1. Hypothetical *Cicindela* species illustrating elytral characters: A) punctures along suture, B) middle band, C) posterior lateral emargination, D) serrulate apical margin, E) apical spine, F) apical lunule, G) apical dot, H) marginal line, I) humeral lunule, J) basal dot.

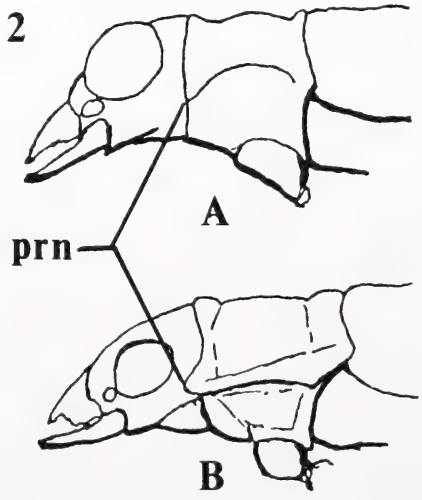


FIGURE 2. Anterior angles of pronotum (prn) of *Cicindela* (A) and *Megacephala* (B) in a lateral view of the head and thorax.

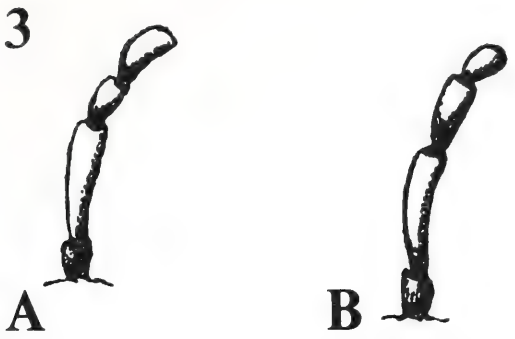


FIGURE 3. Maxillary palps of *Cicindela* (A) and *Megacephala* (B).

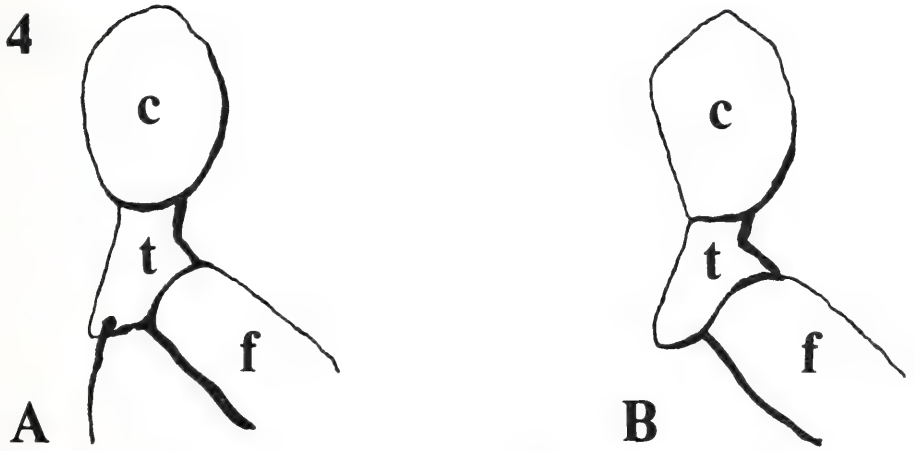


FIGURE 4. Ventral view of leg showing subapical sets on trochanter (A) and showing absence of subapical seta (B); c = coxa, t = trochanter, f = femur. (after Willis, 1968)

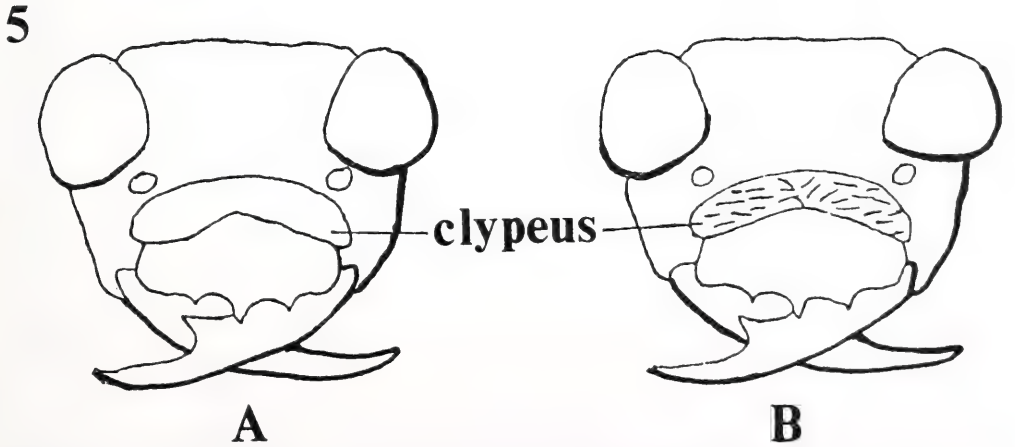


FIGURE 5. Frontal view of head showing clypeus—(A) glabrous (no setae); (B) clothed with decumbent setae.

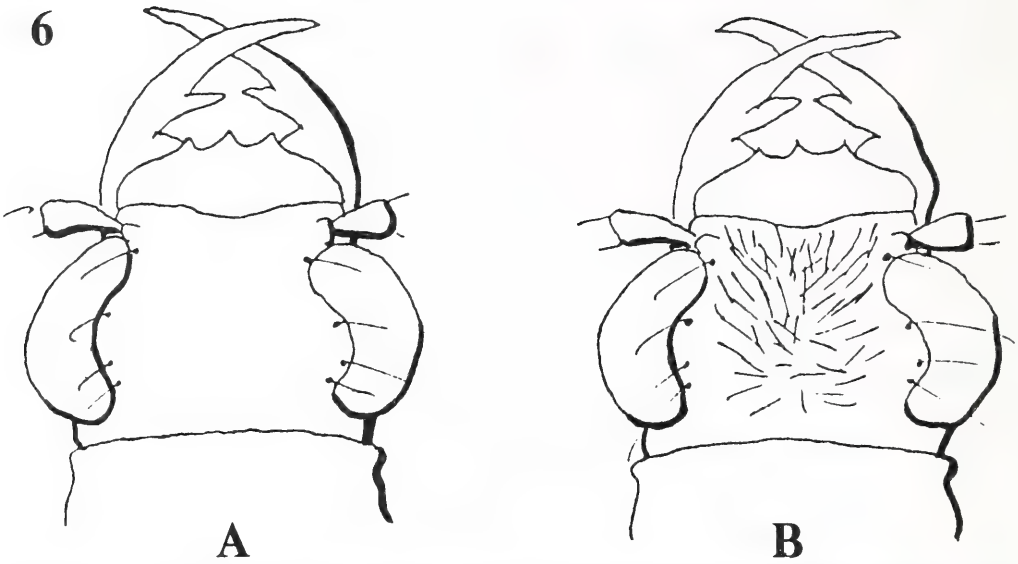


FIGURE 6. Dorsal view of head, frons—(A) glabrous with supraorbital setae only; (B) clothed with setae.

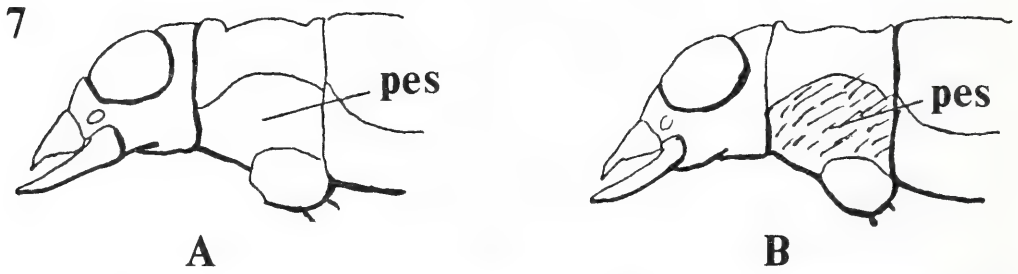


FIGURE 7. Lateral view of head and thorax showing character of proepisternum—(A) glabrous; (B) setose; pes = proepisternum.

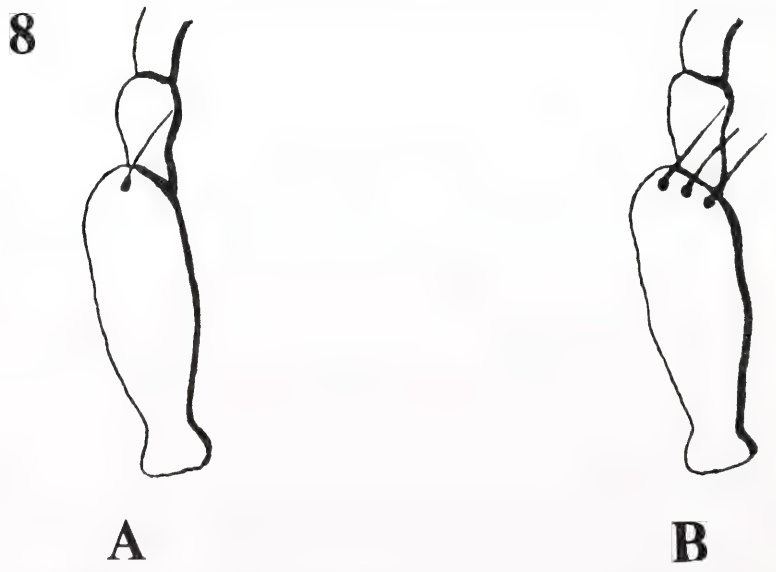


FIGURE 8. Mesal aspect of basal antennal segments showing the character of the setae on the first segment—(A) one sensory seta; (B) three or more sensory setae. (after Willis, 1968)

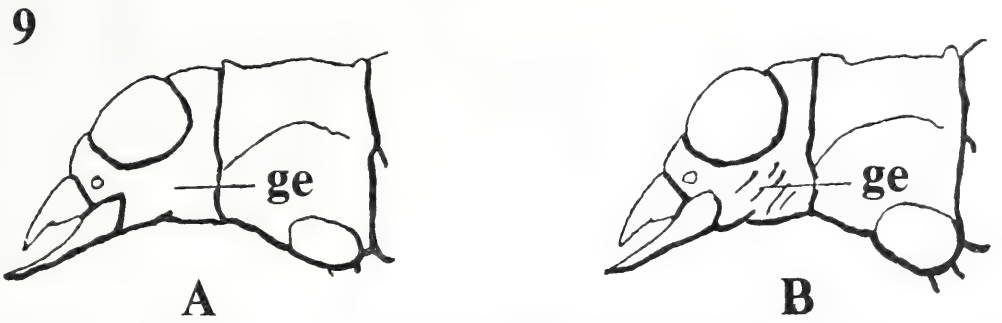


FIGURE 9. Lateral view of head and thorax showing character of gena—(A) glabrous; (B) with setae; ge = gena.

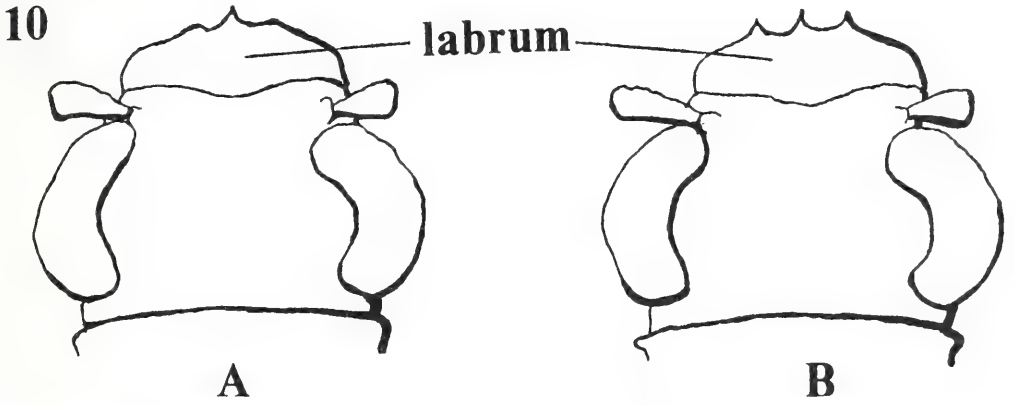


FIGURE 10. Dorsal view of head showing character of labrum—(A) unidentate (Note: rounded bulges are not considered teeth); (B) tridentate.

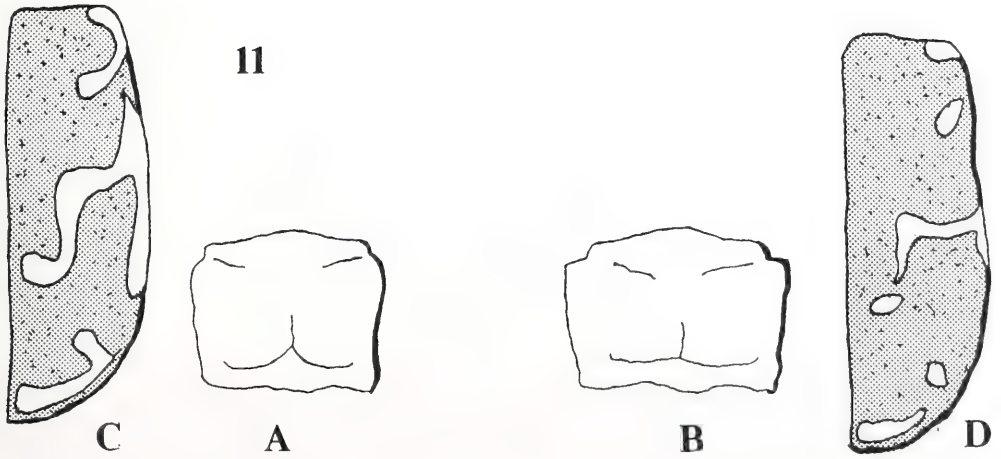
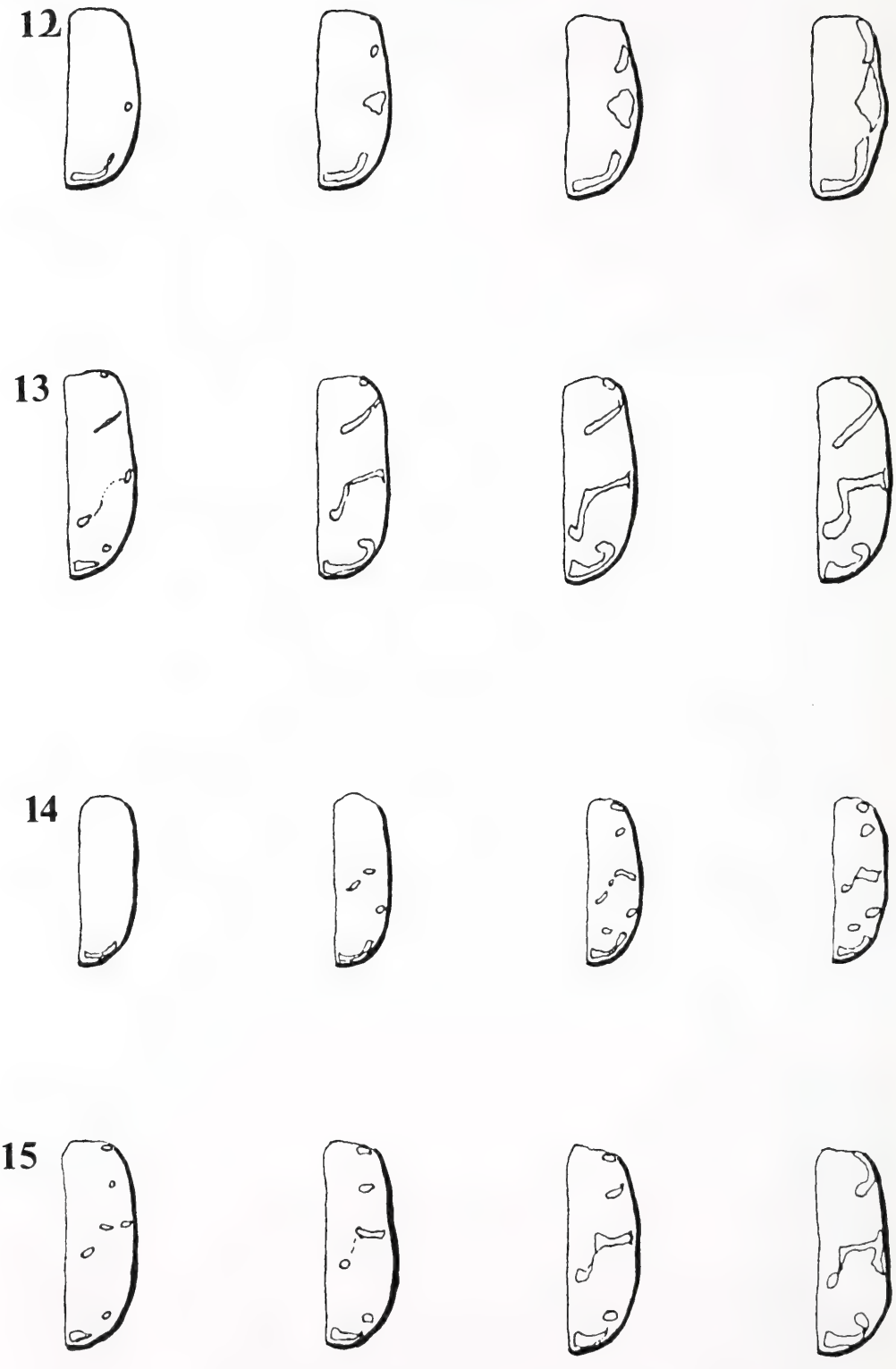
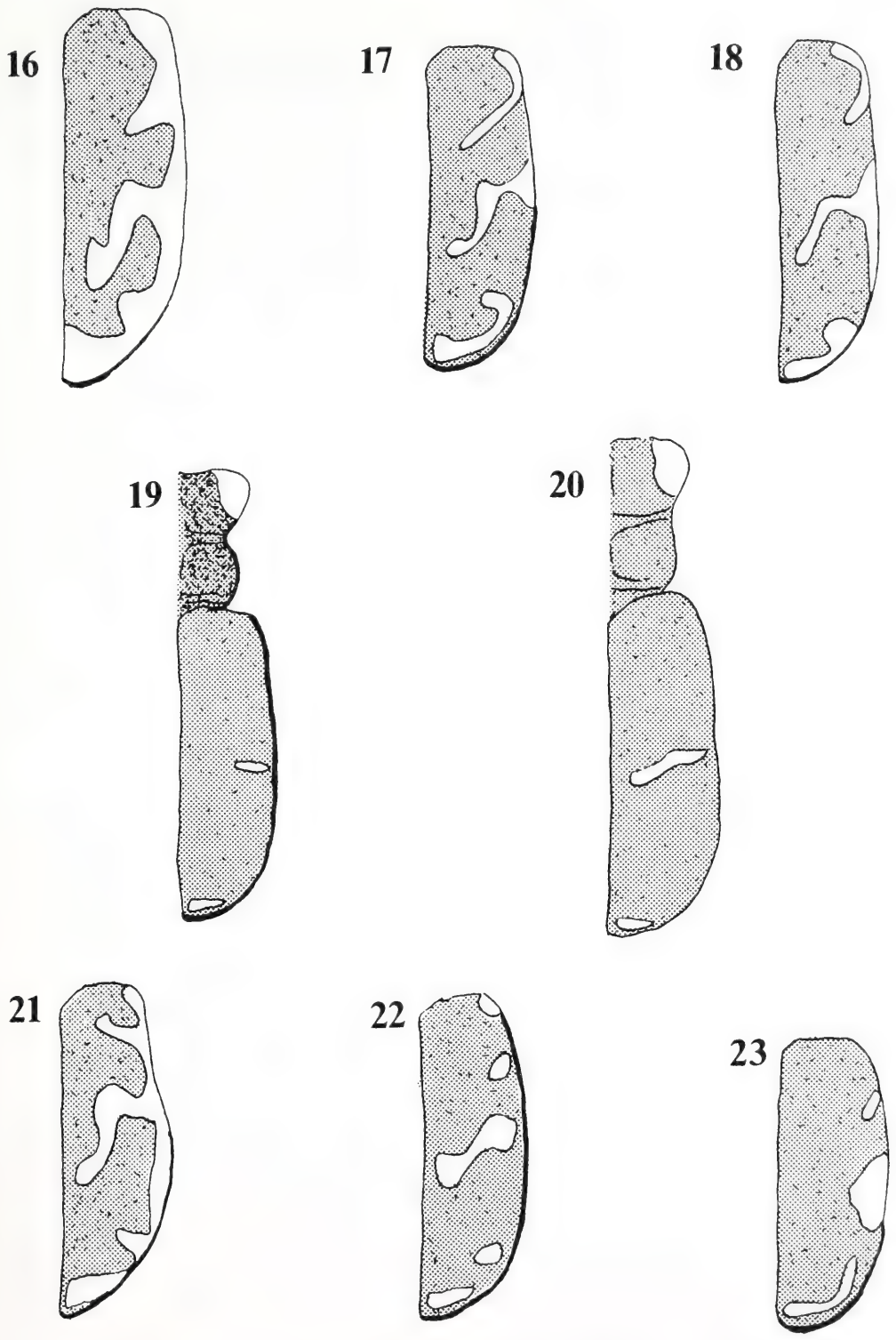


FIGURE 11. Pronota of: A) *Cicindela repanda* showing relatively narrow width and rounded front angles; B) *C. duodecimguttata* showing broader width and acute front angles. (after Willis, 1968) Right elytron of C) *C. repanda*; D) *C. duodecimguttata*.



FIGURES 12-15. Right elytra of 12) *Cicindela scutellaris lecontei*, 13) *C. tranquebarica*, 14) *C. rufiventris*, and 15) *C. duodecimguttata* showing degree of variation in maculation.



FIGURES 16-23. Right elytra of 16) *Cicindela formosa generosa*, 17) *C. tranquebarica*, 18) *C. ancocisconensis*, 19) *C. splendida*, 20) *C. purpurea*. 21) *C. hirticollis*. 22) *C. limbalis*. 23) *C. scutellaris lecontei*.

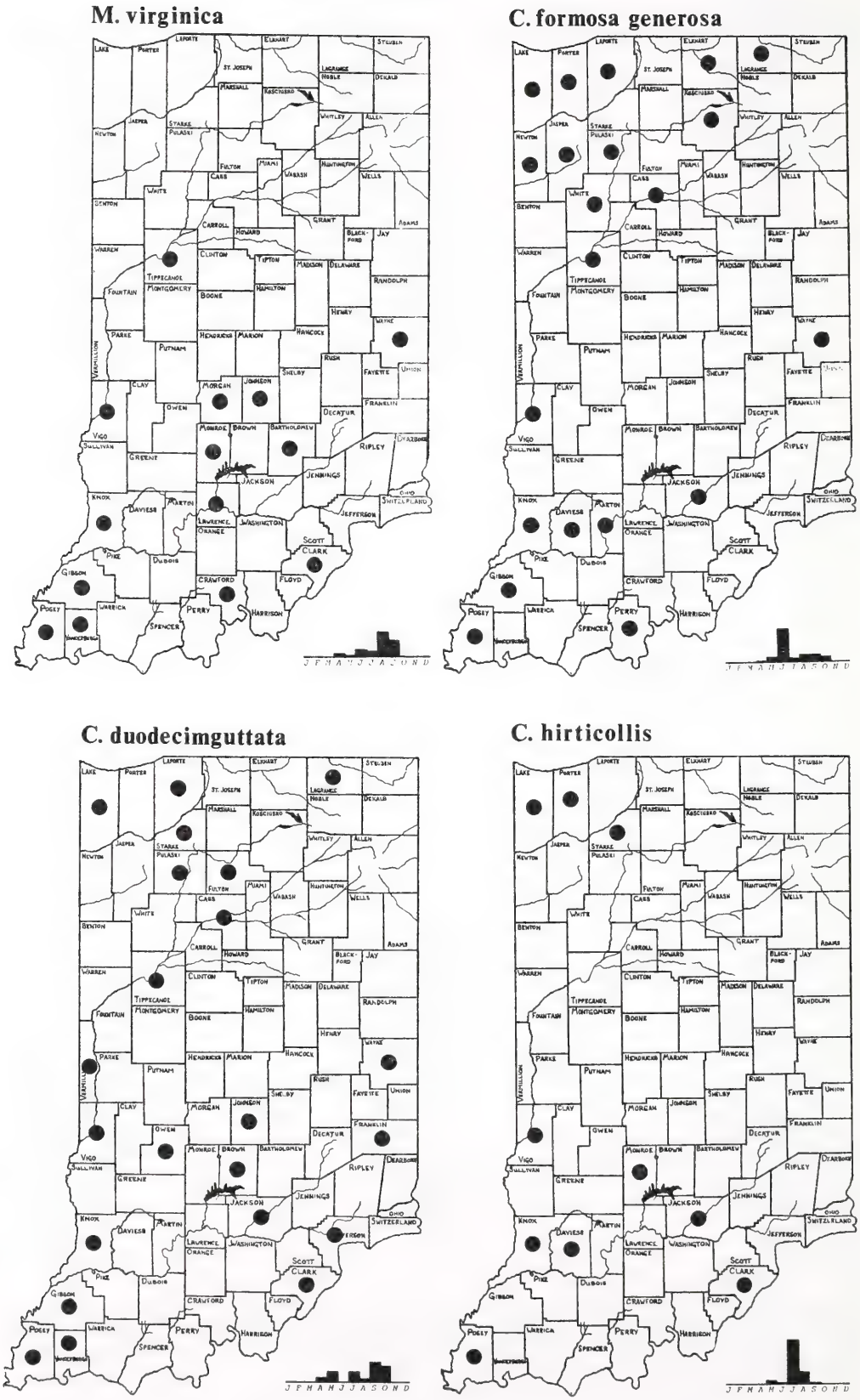


FIGURE 24. Distribution of *Megacephala virginica, Cicindela formosa generosa, C. duodecimguttata*, and *C. hirticollis*.

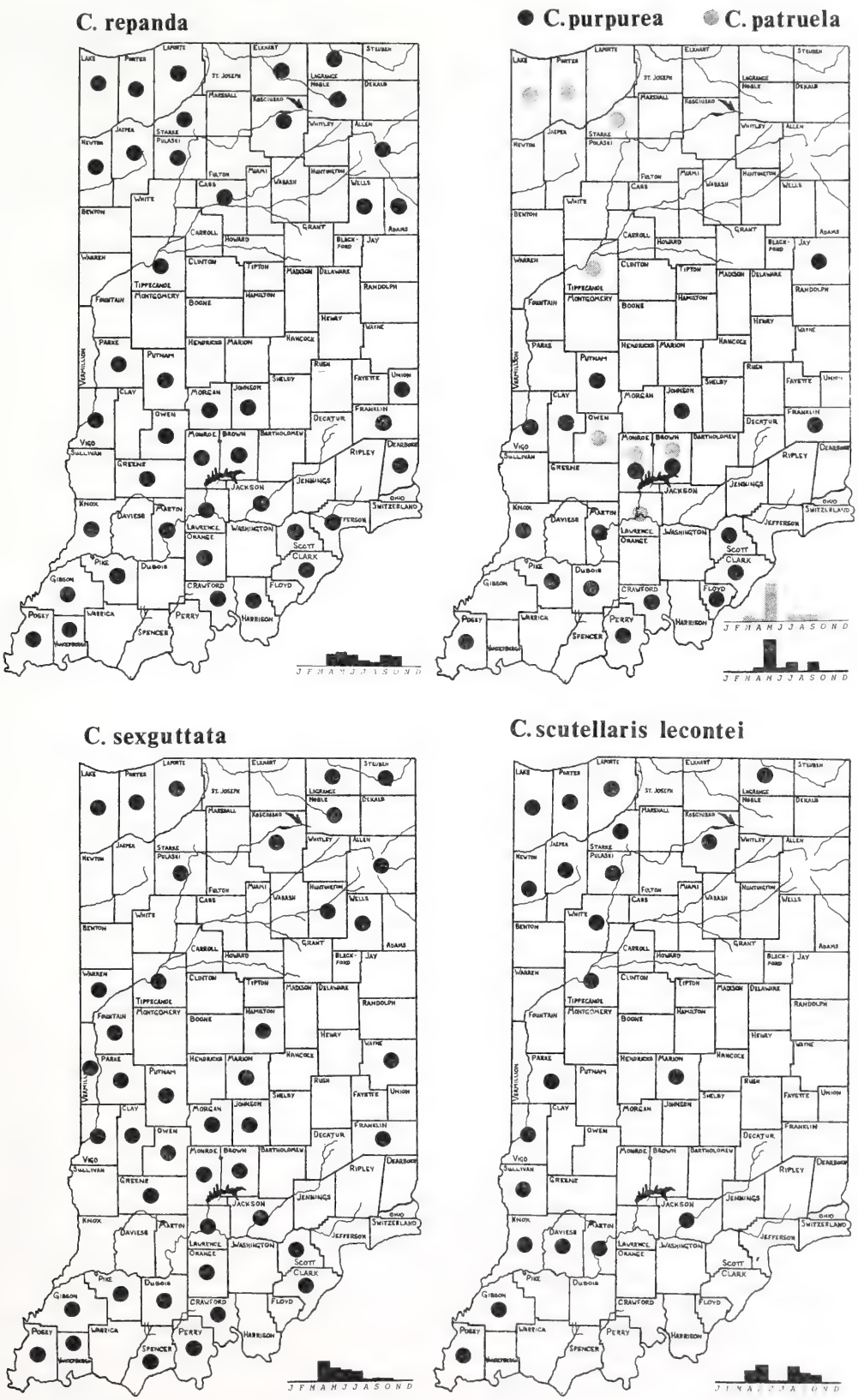


FIGURE 25. Distribution of *Cicindela repanda, C. purpurea, C. patruela, C. sexguttata*, and *C. scutellaris lecontei*.

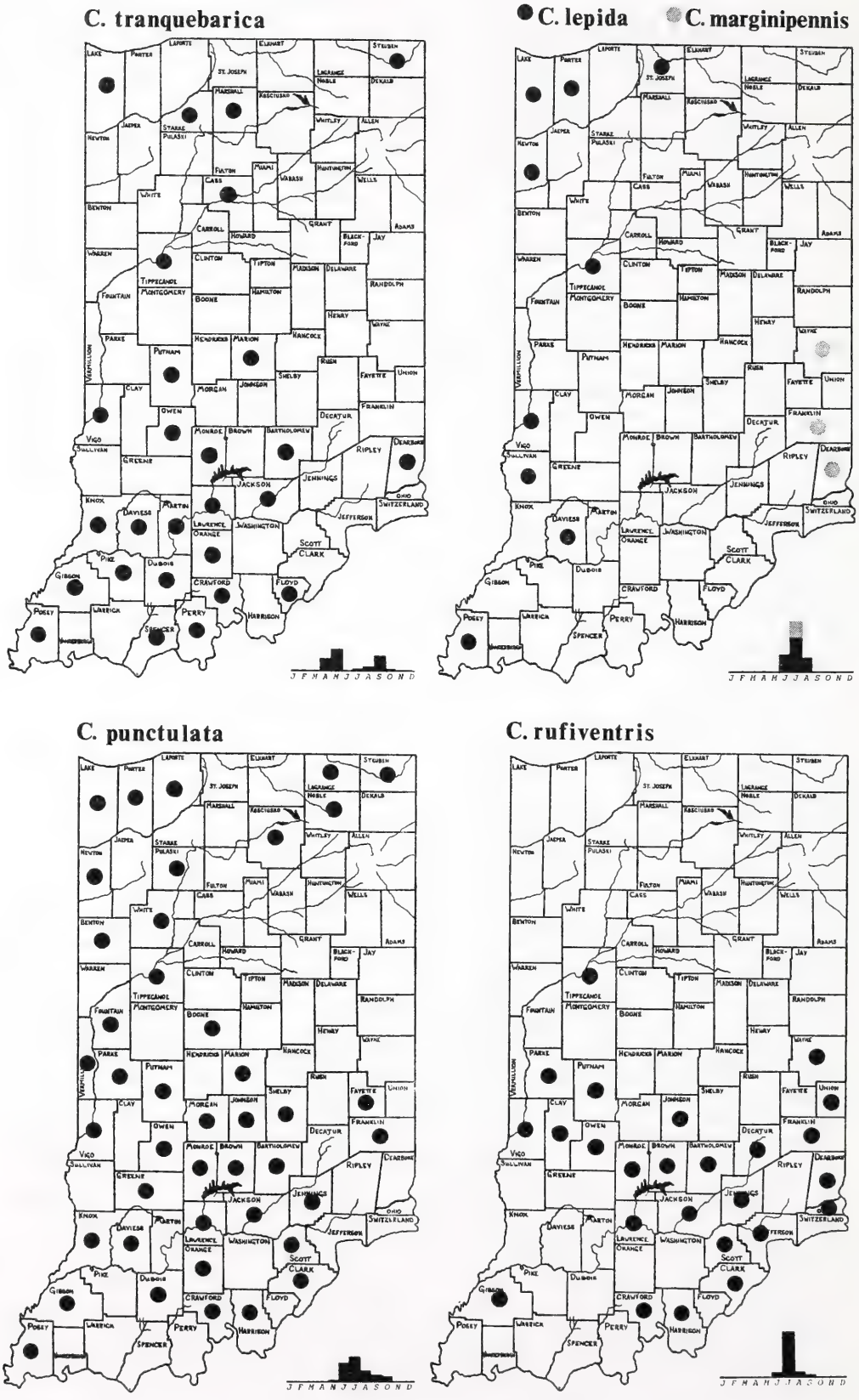


FIGURE 26. Distribution of *Cicindela tranquebarica, C. lepida, C. marginipennis, C. punctulata,* and *C. rufiventris.*

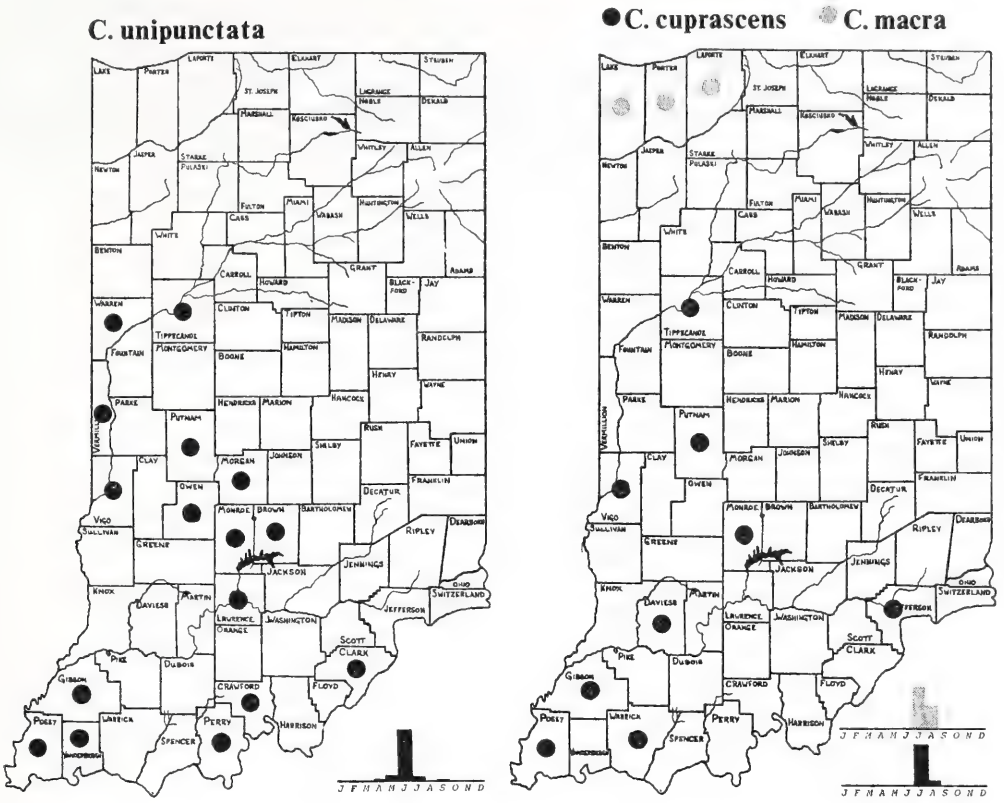


FIGURE 27. Distribution of *Cicindela unipunctata, C. cuprascens*, and *C. macra*.

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An Historical Analysis of Periodical Cicadas in Indiana (Homoptera:Cicadidae)

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Recent studies on the periodical cicadas have provided evidence that these insects may not be as punctual as previously thought. Dybas (1969) and Young (1971, 1975) have documented 17-year cicadas that emerged four years ahead of schedule. In the Greater Cincinnati region, a significant number of cicadas emerged in 1983, four years ahead of the expected major emergence of brood X in 1987. In order to understand the importance of four year accelerations on the evolution of 17-year broods, I examined the historical records of the periodical cicadas in Indiana.

Indiana provides an ideal site for the study of the recent emergence history of the periodical cicadas. The earliest periodical cicada record in Indiana dates back to 1834 with the emergence of brood X. Since then the cicadas' early distributions in the state have been closely studied by Amos W. Butler (1885), and F. M. Webster (1892, 1898). Their work was tabulated along with other Indiana records (Hahn 1905) and published in the Insect Pest Survey Bulletins of the 1930s (Anonymous 1932, 1933, 1936, 1937, 1939, 1940; Hyslop 1935, 1940). Howard Deay (1953), J. J. Davis (1933, 1935, 1954), D. W. Hamilton (1950), M. L. Cleveland (Hamilton and Cleveland 1964), and Frank N. Young (1958, 1971, 1975; Young and Zimmerman 1977) updated this earlier work. The result is probably one of the most complete records of the history of the periodical cicadas in any state.

A major problem encountered with the use of historical records results from errors in assigning brood designations introduced by the original observers in the early surveys. In 1868, 1872, 1881, 1885, and 1898 (Table 1) there were simultaneous

Table 1

Brood	17 year cicada emergence years
VI	1830, 1847, 1864, 1881, 1898, 1915, 1932, 1949, 1966, 1983
X	1834, 1851, 1868, 1885, 1902, 1919, 1936, 1953, 1970, 1987
XIII	1837, 1854, 1871, 1888, 1905, 1922, 1939, 1956, 1973, 1990
XIV	1838, 1855, 1872, 1889, 1906, 1923, 1940, 1957, 1974, 1991
Brood	**13 year cicada emergence years**
XIX	1842, 1855, 1868, 1881, 1894, 1907, 1920, 1933, 1946, 1959, 1972, 1985
XXIII	1846, 1859, 1872, 1885, 1898, 1911, 1924, 1937, 1950, 1963, 1976, 1989

emergences of 17-year and 13-year cicada broods and the early researchers' attempts to designate broods resulted in erroneous interpretations. Another concern arising from the use of historical records is that the records may be of annual cicadas rather than of periodical cicadas. Many of these records are not supported by voucher specimens. In an attempt to remove these errors, 879 cicada records were placed into a database recording the county, town, and year of emergence. Appendix one contains the historical records of the periodical cicadas in Indiana. To verify the nineteenth century county records of cicadas, the database was searched to find consecutive emergences of periodical cicadas in the same counties and towns during expected emergence years. The confirmation of a 13-year or a 17-year emergence pattern permitted an accurate brood designation for those years when Indiana witnessed simultaneous 13- and 17-year cicadas. Moreover, of the 879 emergence records, only

9 reports were in years that do not correspond to expected broods or 4 year accelerations from 17-year broods. This 1.1% error suggests that the historical records are indeed quite accurate. The results confirmed the presence of broods X, XIII, XIV, and XXIII in Indiana in the nineteenth century. Historical records could not confirm the occurrence of brood XIX in Indiana verifying the recent survey conducted by Young (1975).

The results of the database search are summarized in figures 1 through 8. Figure 1 is the historical record of brood XXIII, a 13 year brood, in the nineteenth century. This map represents only those counties in which there were three consecutive emergences 13 years apart in brood XXIII years. Interestingly, the most recent survey by Young (1977) indicates that the distribution of brood XXIII (figure 2) has not changed over the past century.

Brood XIII, a 17-year brood, occurs in just three counties in the northwest portion of the state. It was first recorded in Lake, LaPorte, and Porter (figure 3) counties in 1888 and was most recently documented there in 1956.

The 17-year broods XIV, X, and VI present a more dynamic picture of brood evolution. The nineteenth century records (including 1902) indicate brood X had consistently emerged in all but three of Indiana's 92 counties (figure 4). The most recent survey of brood X (Young and Kritsky 1990) reveals that it now occurs in 64 counties in two sections of the state (figure 5). Brood XIV likewise has greatly reduced its distribution over the past century. Brood XIV in the nineteenth century (including 1906) was repeatedly observed in 23 counties (figure 6), but Young (1975) reported that XIV is now found in only 5 counties (figure 7).

Brood VI, although reported in Indiana since 1898, is not an established brood. All the records of this brood are scattered (figure 8) and are based on only a few individuals. Brood VI, a designation indicating emergence four years before brood X, may indeed represent four year accelerations from brood X. All of the counties where brood VI is recorded are counties in which there was a heavy population of brood X thirteen years prior to the emergence of brood VI.

Four year accelerations have been the focus of several studies. Lloyd and Dybas (1966), Lloyd and White (1976), and Lloyd, Kritsky and Simon (1983) have proposed models to account for the phenomenon of four year accelerations. Indeed, the Indiana record of broods XIV, X, and VI fits the overcrowding hypothesis proposed by Lloyd and White (1976) for the evolution of the 17-year broods.

Lloyd and White (1976) proposed that brood XIV is ancestral to X which is ancestral to VI. They predict that in regions where two broods occur together, the lagging brood XIV will eventually disappear because it is losing individuals to 4-year accelerations. These individuals will emerge during brood X years causing it to increase. Once brood X is established, then the process can begin anew with four year accelerations of brood X giving rise to periodical cicada emergences which would be designated brood VI. These brood VI emergences will initially be too light to satiate predators, and so to establish brood VI. This fits the history of the broods in Indiana over the past 157 years and accounts for the current distributions of broods XIV and X and the reports of scattered records of VI.

For the Lloyd and White (1976) model to work, four year accelerations should be common. Indeed, in the greater Cincinnati region of southeastern Indiana, southwestern Ohio, and northern Kentucky, brood X has produced four year accelerations in 1898, 1932, 1966, and 1983. An analysis of the Indiana periodical cicada database shows that over 20 percent of the brood X counties have apparently given rise to four year accelerations. A similar survey of the historical records of the periodical cicadas in Ohio reveals that over 30 percent of the brood X counties have recorded cicadas thirteen years after a brood X appearance.

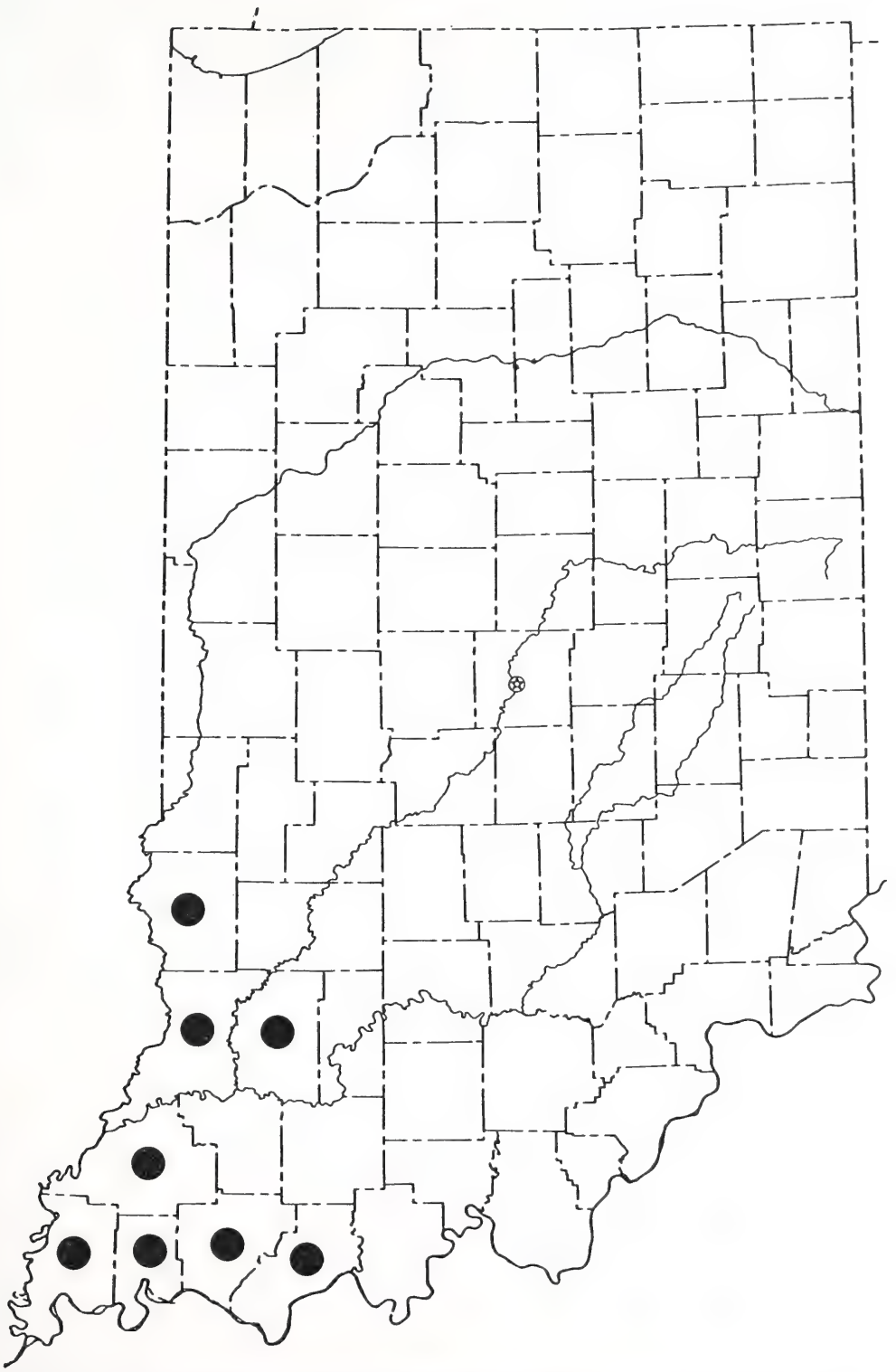

FIGURE 1. The nineteenth century distribution of the 13-year brood XXIII.

The relatively common occurrence of four year accelerations and their impact on the populations of both the lagging and accelerating broods suggest that the periodical cicada broods are evolving rapidly with significant changes in distribution and density occurring in a matter of decades.

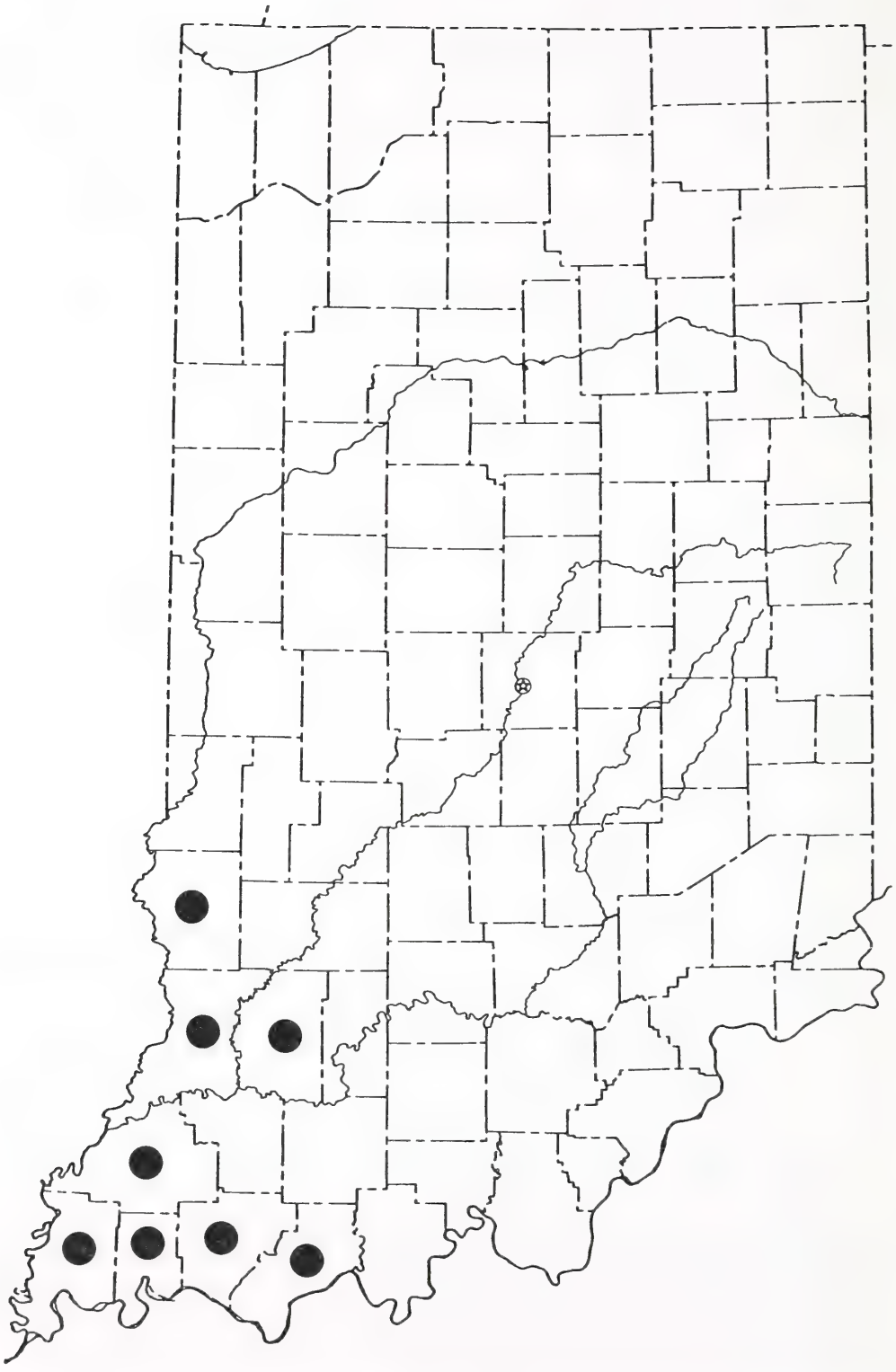

FIGURE 2. The distribution of brood XXIII in 1976 (After Young and Zimmerman 1977).

FIGURE 3. The distribution of the 17-year brood XIII.

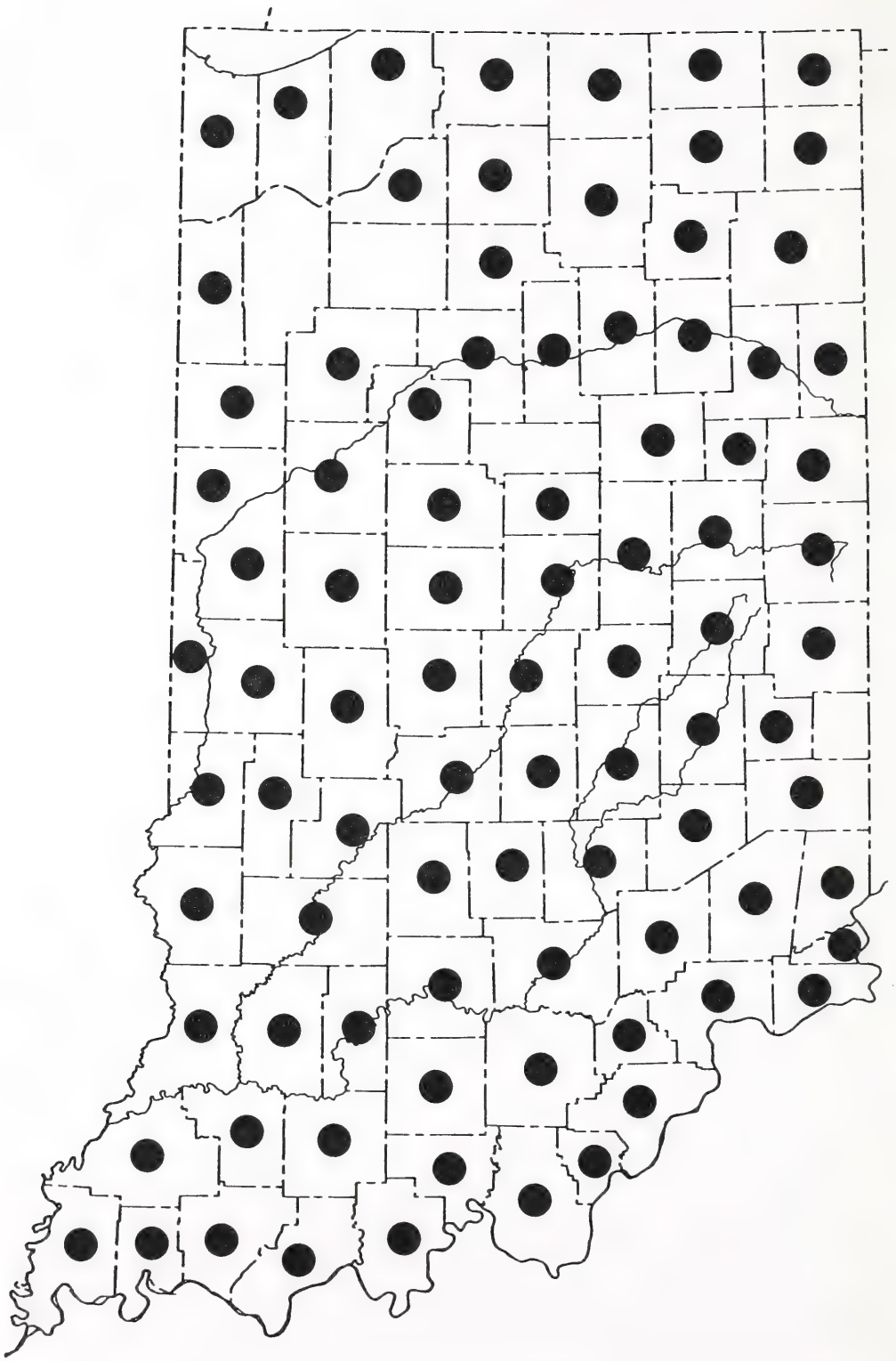

FIGURE 4. The nineteenth century distribution (including 1902) of the 17-year brood X.

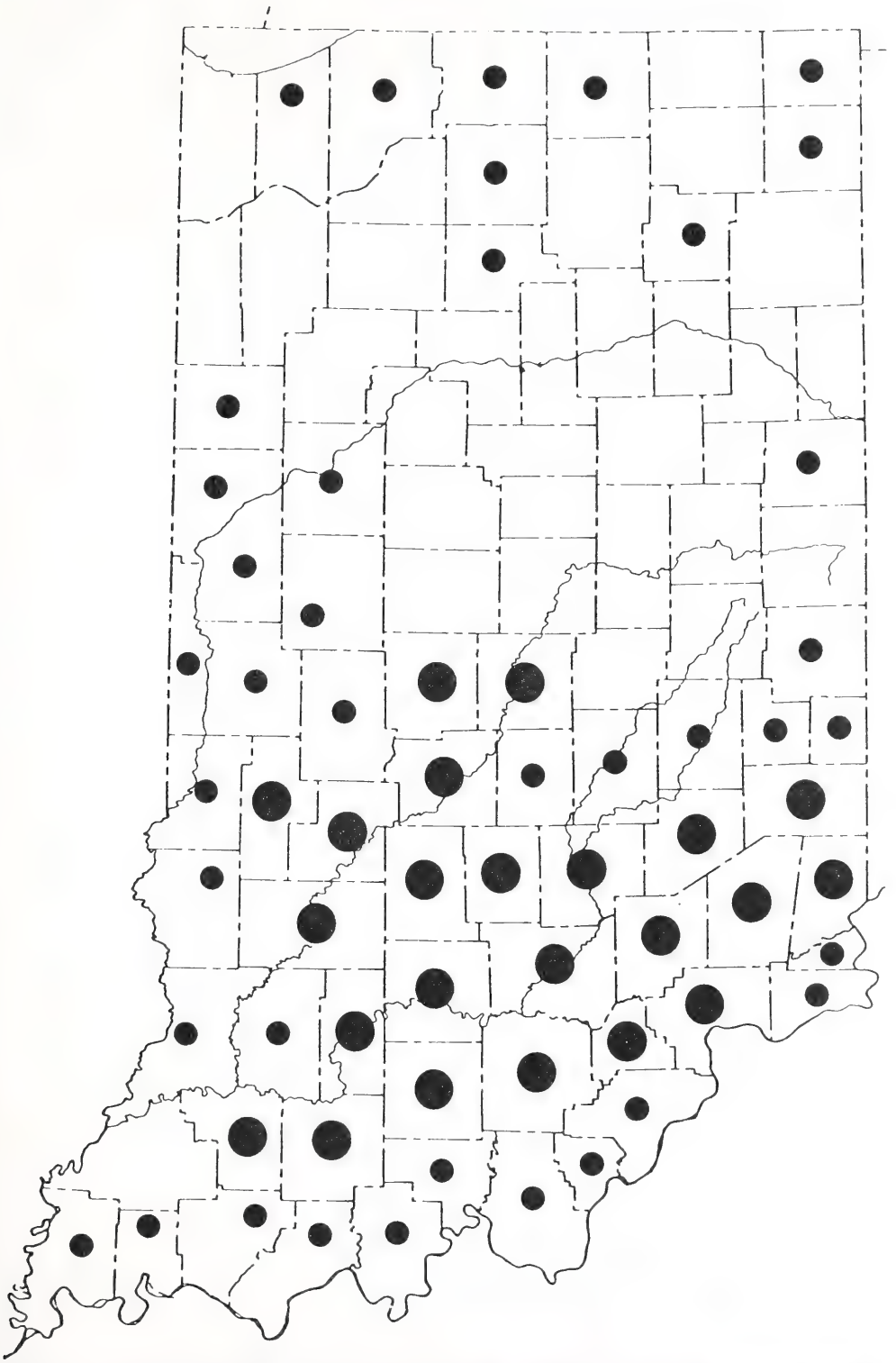

FIGURE 5. The distribution of brood X in 1987 (After Young and Kritsky 1988).

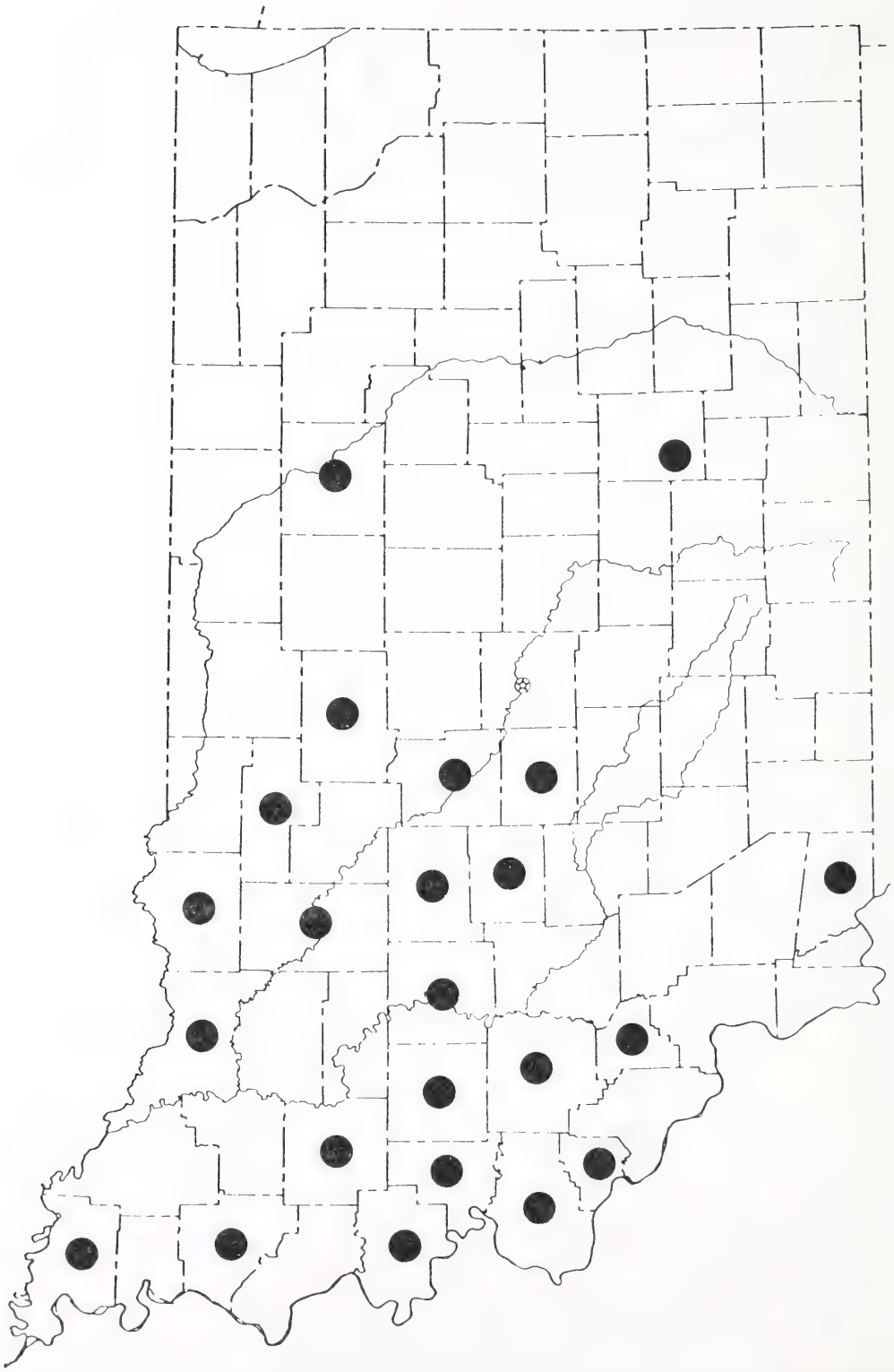

FIGURE 6. The nineteenth century distribution (including 1906) of the 17-year brood XIV.

FIGURE 7. The distribution of brood XIV in 1974 (After Young 1975).

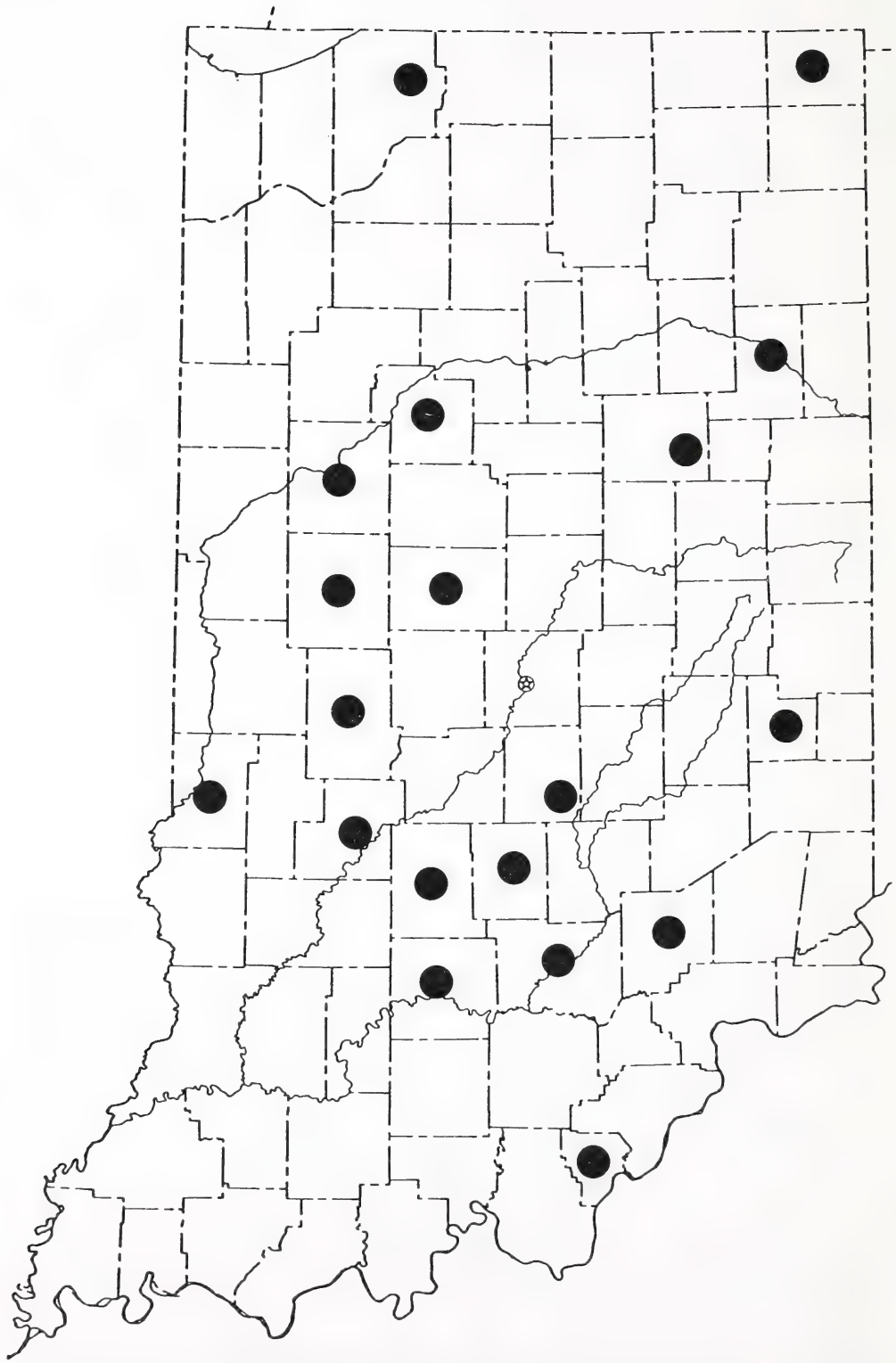

FIGURE 8. Counties reporting probable four year accelerations of brood X, designated brood VI.

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Appendix One
Indiana's Periodical Cicada Records

County	Town	Year
Adams	Berne	1885
Adams	Berne	1902
Adams	—	1919
Allen	Ft. Wayne	1885
Allen	—	1887
Allen	—	1902
Bartholomew	Hope	1885
Bartholomew	Columbus	1902
Bartholomew	Hope	1902
Bartholomew	—	1970
Bartholomew	Azalia	1987
Bartholomew	Columbus	1987
Bartholomew	Elizabethtown	1987
Bartholomew	Hope	1987
Bartholomew	Taylorsville	1987
Benton	Boswell	1885
Benton	Fowler	1902
Benton	Freeland Park	1987
Blackford	—	1868
Blackford	Hartford City	1885
Blackford	Hartford City	1902
Blackford	Montpelier	1902
Boone	—	1885
Boone	Lebanon	1898
Boone	Thorntown	1898
Boone	Lebanon	1902
Boone	Whitestown	1902
Boone	—	1905
Boone	Lebanon	1906
Brown	—	1868
Brown	Ramelton	1885
Brown	—	1889
Brown	Cornelius	1898
Brown	Bean Blossom	1902
Brown	Mount Liberty	1902
Brown	New Bellsville	1902
Brown	Nashville	1906
Brown	Brown Co. St. Park	1936
Brown	—	1940
Brown	—	1957
Brown	Nashville	1957
Brown	Nashville	1957
Brown	—	1970
Brown	—	1974
Brown	Brown Co St Park	1987
Brown	Nashville	1987
Carroll	—	1885
Carroll	Rockfield	1898
Carroll	Cutler	1902
Carroll	Delphi	1902
Carroll	Rockfield	1902
Carroll	Delphi	1906
Carroll	Delphi	1919

County	Town	Year
Cass	—	1885
Cass	Logansport	1902
Clark	New Providence	1834
Clark	Charlestown	1851
Clark	New Providence	1851
Clark	Charlestown	1868
Clark	New Providence	1868
Clark	Charlestown	1885
Clark	Henryville	1885
Clark	New Providence	1885
Clark	Otto	1885
Clark	—	1889
Clark	Jeffersonville	1902
Clark	Otto	1902
Clark	Sellersburg	1902
Clark	Utica	1902
Clark	—	1970
Clark	—	1987
Clay	—	1868
Clay	—	1872
Clay	Ashboro	1885
Clay	Cory	1885
Clay	—	1889
Clay	Bowling Green	1902
Clay	Brazil	1902
Clay	Cory	1902
Clay	—	1970
Clinton	Frankfort	1885
Clinton	Frankfort	1902
Clinton	Rossville	1902
Crawford	Pilot Knob	1851
Crawford	Pilot Knob	1868
Crawford	—	1872
Crawford	Pilot Knob	1885
Crawford	—	1889
Crawford	Pilot Knob	1902
Crawford	West Fork	1902
Crawford	—	1957
Crawford	—	1974
Crawford	Eckerty	1987
Crawford	Marengo	1987
Crawford	Milltown	1987
Crawford	Taswell	1987
Daviess	Cumback	1868
Daviess	Marsh	1885
Daviess	Washington	1885
Daviess	—	1889
Daviess	—	1898
Daviess	Cannelburg	1902
Daviess	Marsh	1902
Daviess	Washington	1902
Daviess	—	1924
Daviess	—	1963
Daviess	—	1970
Daviess	—	1976
Daviess	—	1987
Dearborn	Aurora	1834

County	Town	Year
Dearborn	—	1843
Dearborn	Aurora	1851
Dearborn	—	1860
Dearborn	Aurora	1868
Dearborn	Guilford	1868
Dearborn	Aurora	1885
Dearborn	Cochran	1885
Dearborn	Guilford	1885
Dearborn	—	1889
Dearborn	—	1894
Dearborn	Aurora	1902
Dearborn	Lawrenceburg	1902
Dearborn	Sparta	1902
Dearborn	Wright Corners	1902
Dearborn	Yorkville	1902
Dearborn	—	1906
Dearborn	—	1919
Dearborn	—	1970
Dearborn	Aurora	1987
Dearborn	Dillsboro	1987
Dearborn	Lawrenceburg	1987
Decatur	Greensburg	1868
Decatur	—	1885
Decatur	Alert	1885
Decatur	Greensburg	1885
Decatur	Letts	1885
Decatur	Westport	1885
Decatur	Adams	1902
Decatur	Alert	1902
Decatur	Greensburg	1902
Decatur	Letts	1902
Decatur	—	1970
Decatur	Greensburg	1987
Decatur	Westport	1987
DeKalb	Waterloo	1885
DeKalb	Stafford Twp.	1902
DeKalb	Waterloo	1902
DeKalb	—	1919
DeKalb	Waterloo	1987
Delaware	—	1868
Delaware	Muncie	1885
Delaware	Muncie	1902
Dubois	Jasper	1851
Dubois	Jasper	1868
Dubois	Ellsworth	1885
Dubois	Jasper	1885
Dubois	—	1889
Dubois	Crystal	1902
Dubois	Ellsworth	1902
Dubois	Johnsburg	1902
Dubois	—	1906
Dubois	Jasper	1919
Dubois	—	1970
Dubois	—	1987
Elkhart	Middlebury	1885
Elkhart	Elkhart	1902
Elkhart	Goshen	1902

County	Town	Year
Elkhart	Elkhart	1987
Elkhart	Goshen	1987
Elkhart	Middlebury	1987
Elkhart	Millersburg	1987
Elkhart	Nappanee	1987
Elkhart	NW corner of County	1987
Fayette	Connersville	1868
Fayette	—	1885
Fayette	—	1898
Fayette	Alpine	1902
Fayette	Lyons Station	1902
Fayette	—	1987
Floyd	New Albany	1851
Floyd	New Albany	1868
Floyd	Galena	1885
Floyd	New Albany	1885
Floyd	—	1889
Floyd	—	1898
Floyd	Floyds Knob	1902
Floyd	Galena	1902
Floyd	New Albany	1902
Floyd	—	1906
Floyd	—	1919
Floyd	—	1970
Floyd	Floyds Knob	1987
Floyd	Georgetown	1987
Floyd	Greenville	1987
Floyd	Starlight	1987
Fountain	Covington	1851
Fountain	Covington	1868
Fountain	Covington	1885
Fountain	Hillsboro	1885
Fountain	Kingman	1885
Fountain	Covington	1902
Fountain	Hillsboro	1902
Fountain	Kingman	1902
Fountain	Silverwood	1906
Fountain	—	1911
Fountain	Silverwood	1911
Fountain	—	1970
Fountain	Covington	1987
Fountain	Kingman	1987
Franklin	—	1834
Franklin	—	1851
Franklin	Mount Carmel	1868
Franklin	Peppertown	1868
Franklin	Brookville	1885
Franklin	Mount Carmel	1885
Franklin	Brookville	1889
Franklin	Cedar Grove	1902
Franklin	Laurel	1902
Franklin	Peppertown	1902
Franklin	—	1987
Fulton	Rochester	1868
Fulton	Rochester	1885
Fulton	Leiters Ford	1902
Fulton	Rochester	1902

County	Town	Year
Fulton	Akron	1987
Fulton	Rochester	1987
Gibson	Fransico	1885
Gibson	—	1889
Gibson	Haubstadt	1898
Gibson	Oakland	1898
Gibson	Princeton	1898
Gibson	Fort Branch	1902
Gibson	Haubstadt	1902
Gibson	Oakland City	1902
Gibson	Princeton	1902
Gibson	—	1924
Gibson	—	1963
Gibson	—	1976
Grant	Upland	1851
Grant	Jonesboro	1868
Grant	Marion	1868
Grant	Upland	1868
Grant	Grant	1885
Grant	Jonesboro	1885
Grant	Marion	1885
Grant	Upland	1885
Grant	Southeastern part	1889
Grant	Upland	1898
Grant	Upland	1902
Grant	Southeastern part	1906
Greene	Worthington	1868
Greene	Worthington	1872
Greene	Worthington	1885
Greene	—	1889
Greene	Bloomfield	1902
Greene	Scotland	1902
Greene	—	1919
Greene	—	1970
Greene	Bloomfield	1987
Greene	Jasonville	1987
Greene	Owensburg	1987
Greene	Solsberry	1987
Greene	Worthington	1987
Hamilton	Boxley	1885
Hamilton	Carmel	1885
Hamilton	Carmel	1902
Hamilton	Cicero	1902
Hamilton	Hortonville	1902
Hancock	Philadelphia	1885
Hancock	Greenfield	1902
Harrison	Palmyra	1851
Harrison	Palmyra	1868
Harrison	Palmyra	1885
Harrison	—	1889
Harrison	Palmyra	1902
Harrison	—	1906
Harrison	Corydon	1923
Harrison	Corydon Junction	1923
Harrison	Laconia	1923
Harrison	—	1957
Harrison	—	1957

County	Town	Year
Harrison	Corydon	1957
Harrison	—	1974
Harrison	Corydon	1987
Harrison	Laconia	1987
Harrison	Lanesville	1987
Harrison	Mauckport	1987
Harrison	Middleburg	1987
Harrison	Milltown	1987
Harrison	New Amsterdam	1987
Harrison	Palmyra	1987
Hendricks	Hadley	1834
Hendricks	Hadley	1851
Hendricks	Hadley	1868
Hendricks	Center Valley	1885
Hendricks	Danville	1885
Hendricks	Hadley	1885
Hendricks	Cartersburg	1902
Hendricks	Center Valley	1902
Hendricks	Danville	1902
Hendricks	—	1970
Hendricks	Cartersburg	1987
Hendricks	Clayton	1987
Hendricks	Danville	1987
Hendricks	Hadley	1987
Hendricks	New Winchester	1987
Hendricks	North Salem	1987
Hendricks	Plainfield	1987
Henry	Mechanicsburg	1868
Henry	Spiceland	1868
Henry	Mechanicsburg	1885
Henry	Spiceland	1885
Henry	Middletown	1902
Henry	Mooreland	1902
Henry	Newcastle	1902
Henry	Spiceland	1902
Henry	Spiceland	1919
Howard	—	1902
Huntington	Huntington	1868
Huntington	Huntington	1885
Huntington	Warren	1885
Huntington	Huntington	1902
Jackson	Ewing	1851
Jackson	Ewing	1868
Jackson	Rockford	1868
Jackson	Ewing	1885
Jackson	Medora	1885
Jackson	Rockford	1885
Jackson	Seymour	1885
Jackson	—	1898
Jackson	Brownstown	1902
Jackson	Kurtz	1902
Jackson	Medora	1902
Jackson	Seymour	1902
Jackson	Brownstown	1906
Jackson	—	1970
Jackson	Brownstown	1987
Jackson	Crothersville	1987

County	Town	Year
Jackson	Medora	1987
Jackson	Norman	1987
Jackson	Reddington	1987
Jackson	Seymour	1987
Jackson	Tampico	1987
Jackson	Vallonia	1987
Jasper	—	1885
Jay	Dunkirk	1885
Jay	Dunkirk	1902
Jay	—	1919
Jay	—	1987
Jefferson	Madison	1834
Jefferson	Madison	1851
Jefferson	Madison	1868
Jefferson	Stony Point	1868
Jefferson	Madison	1885
Jefferson	Marble Hill	1885
Jefferson	Stony Point	1885
Jefferson	Deputy	1902
Jefferson	Madison	1902
Jefferson	Madison	1940
Jefferson	—	1970
Jefferson	—	1987
Jennings	North Vernon	1885
Jennings	—	1898
Jennings	Barnes	1902
Jennings	North Vernon	1902
Jennings	Commiskey	1987
Jennings	Hayden	1987
Jennings	North Vernon	1987
Jennings	Scipio	1987
Jennings	Vernon	1987
Johnson	—	1885
Johnson	Franklin	1889
Johnson	Edinburgh	1898
Johnson	Banta	1902
Johnson	Franklin	1902
Johnson	Samaria	1902
Johnson	—	1906
Johnson	—	1970
Johnson	Amity	1987
Johnson	Bargersville	1987
Johnson	Edinburgh	1987
Johnson	Nineveh	1987
Johnson	Trafalger	1987
Knox	—	1842
Knox	—	1859
Knox	Bicknell	1872
Knox	Vincennes	1885
Knox	Vincennes	1889
Knox	Vincennes	1898
Knox	Wheatland	1898
Knox	Bicknell	1902
Knox	Oaktown	1902
Knox	Vincennes	1902
Knox	Wheatland	1902
Knox	Bicknell	1906

County	Town	Year
Knox	Vincennes	1906
Knox	Vincennes	1911
Knox	Vincennes	1924
Knox	Bicknell	1936
Knox	Vincennes	1937
Knox	—	1950
Knox	—	1963
Knox	—	1970
Knox	—	1976
Knox	Vincennes	1987
Kosciusko	Syracuse	1851
Kosciusko	Syracuse	1868
Kosciusko	Leesburgh	1885
Kosciusko	Milford	1885
Kosciusko	Sidney	1885
Kosciusko	Syracuse	1885
Kosciusko	Milford	1902
Lagrange	Lagrange	1868
Lagrange	Lagrange	1885
Lake	—	1872
Lake	Hobart	1885
Lake	Lowell	1885
Lake	—	1888
Lake	Crown Point	1935
Lake	—	1939
Lake	—	1956
Lake	Crown Point	1956
LaPorte	—	1885
LaPorte	—	1888
LaPorte	Rolling Prairie	1898
LaPorte	LaPorte	1902
LaPorte	—	1939
LaPorte	—	1956
LaPorte	—	1956
LaPorte	—	1987
LaPorte	Stillwell	1987
Lawrence	Erie	1868
Lawrence	Mitchell	1868
Lawrence	Erie	1885
Lawrence	Mitchell	1885
Lawrence	—	1889
Lawrence	Beford	1902
Lawrence	Fort Ritner	1902
Lawrence	Zelma	1902
Lawrence	—	1906
Lawrence	—	1932
Lawrence	—	1940
Lawrence	—	1957
Lawrence	—	1957
Lawrence	—	1970
Lawrence	Bedford	1987
Lawrence	Heltonville	1987
Lawrence	Mitchell	1987
Lawrence	Springville	1987
Madison	—	1834
Madison	—	1851
Madison	—	1868

County	Town	Year
Madison	Lapel	1885
Madison	Pendleton	1885
Madison	Anderson	1902
Madison	Frankton	1902
Madison	Lapel	1902
Marion	Indianapolis	1834
Marion	Indianapolis	1851
Marion	New Augusta	1851
Marion	New Augusta	1868
Marion	Indianapolis	1885
Marion	New Augusta	1885
Marion	New Augusta	1885
Marion	Acton	1902
Marion	Broad Ripple	1902
Marion	Clermont	1902
Marion	Indianapolis	1902
Marion	New Augusta	1902
Marion	—	1970
Marion	Acton	1987
Marion	Broad Ripple	1987
Marion	Clermont	1987
Marion	Indianapolis	1987
Marshall	—	1902
Marshall	Argos	1987
Marshall	Culver	1987
Marshall	Plymouth	1987
Martin	Trinity Springs	1868
Martin	Trinity Springs	1885
Martin	Indian Springs	1902
Martin	Loogootee	1902
Martin	—	1970
Martin	Loogootee	1987
Martin	Shoals	1987
Miami	—	1885
Miami	Gilead	1902
Monroe	Stanford	1851
Monroe	Bloomington	1868
Monroe	Stanford	1868
Monroe	Bloomington	1885
Monroe	Stanford	1885
Monroe		1889
Monroe	Bloomington	1902
Monroe	Stanford	1902
Monroe	Unionville	1902
Monroe	—	1906
Monroe	Bloomington	1966
Monroe	—	1970
Monroe	Bloomington	1987
Montgomery	New Ross	1851
Montgomery	Crawfordsville	1868
Montgomery	New Ross	1868
Montgomery	Crawfordsville	1885
Montgomery	Mace	1885
Montgomery	New Ross	1885
Montgomery	—	1898
Montgomery	Browns Valley	1902
Montgomery	Crawfordsville	1902

County	Town	Year
Montgomery	Linden	1902
Montgomery	—	1970
Montgomery	Alamo	1987
Morgan	Martinsville	1834
Morgan	Martinsville	1851
Morgan	Martinsville	1868
Morgan	Martinsville	1885
Morgan	Morgantown	1885
Morgan	—	1889
Morgan	Martinsville	1902
Morgan	Monrovia	1902
Morgan	—	1906
Morgan	—	1970
Morgan	—	1974
Newton	Kentland	1868
Newton	Kentland	1885
Noble	—	1885
Noble	Rome City	1902
Ohio	Bascom	1902
Ohio	Glue	1902
Ohio	Rising Sun	1902
Ohio	Bascom	1919
Ohio	Patriot	1987
Orange	—	1868
Orange	French Lick	1885
Orange	Orleans	1885
Orange	—	1889
Orange	Fargo	1902
Orange	French Lick	1902
Orange	Paoli	1902
Orange	West Baden	1902
Orange	—	1906
Orange	French Lick	1919
Orange	Orleans	1936
Orange	—	1938
Orange	—	1957
Orange	Orleans	1957
Orange	—	1970
Orange	French Lick	1987
Orange	Orleans	1987
Orange	Paoli	1987
Owen	Spencer	1851
Owen	Spencer	1868
Owen	Spencer	1885
Owen	—	1898
Owen	Quincy	1902
Owen	Spencer	1902
Owen	Vandalia	1902
Owen	—	1970
Owen	—	1987
Parke	Rockville	1868
Parke	Rockville	1885
Parke	Judson	1902
Parke	Marshall	1902
Parke	Rockville	1902
Parke	—	1970
Parke	—	1987

County	Town	Year
Perry	—	1868
Perry	Mount Pleasant	1885
Perry	Rome	1885
Perry	Bristow	1902
Perry	Magnet	1902
Perry	Mount Pleasant	1902
Perry	Rome	1902
Perry	Troy	1902
Perry	Dexter	1906
Perry	Rome	1919
Perry	—	1957
Perry	—	1970
Perry	—	1974
Perry	Cannelton	1987
Perry	Tell City	1987
Pike	Petersburg	1868
Pike	Petersburg	1885
Pike	—	1889
Pike	Petersburg	1902
Pike	Velpen	1902
Pike	Petersburg	1919
Pike	—	1924
Pike	—	1970
Pike	—	1987
Porter	—	1888
Porter	—	1902
Porter	—	1939
Porter	—	1956
Porter	—	1956
Porter	Ogden Dunes	1987
Porter	Portage	1987
Posey	—	1859
Posey	New Harmony	1859
Posey	New Harmony	1872
Posey	New Harmony	1872
Posey	—	1877
Posey	New Harmony	1885
Posey	New Harmony	1885
Posey	—	1889
Posey	Mount Vernon	1894
Posey	Mount Vernon	1898
Posey	New Harmony	1898
Posey	Wadesville	1898
Posey	Wadesville	1902
Posey	Goose Pond	1911
Posey	—	1924
Posey	—	1963
Posey	—	1976
Posey	Griffin	1987
Posey	Wadesville	1987
Pulaski	—	1902
Putnam	Greencastle	1834
Putnam	Greencastle	1851
Putnam	Putnamville	1851
Putnam	Greencastle	1868
Putnam	Putnamville	1868
Putnam	Greencastle	1885

County	Town	Year
Putnam	Putnamville	1885
Putnam	Greencastle	1889
Putnam	—	1898
Putnam	Fincastle	1902
Putnam	Greencastle	1902
Putnam	Putnamville	1902
Putnam	—	1906
Putnam	—	1970
Putnam	Cloverdale	1987
Putnam	Greencastle	1987
Putnam	Putnamville	1987
Putnam	Reelsville	1987
Randolph	Trenton	1885
Randolph	Winchester	1885
Randolph	Modoc	1902
Randolph	Ridgeville	1902
Ripley	Sunman	1934
Ripley	Sunman	1851
Ripley	Milan	1868
Ripley	Sunman	1868
Ripley	Sunman	1868
Ripley	Hyde	1885
Ripley	Milan	1885
Ripley	Spades	1885
Ripley	Sunman	1885
Ripley	Sunman	1885
Ripley	Benham	1902
Ripley	Cross Plains	1902
Ripley	Gaff	1902
Ripley	Hyde	1902
Ripley	Napoleon	1902
Ripley	Springdale	1902
Ripley	Sunman	1902
Ripley	Holton	1906
Ripley	Napoleon	1919
Ripley	—	1970
Ripley	Batesville	1987
Ripley	Cross Plains	1987
Ripley	Elrod	1987
Ripley	Holton	1987
Ripley	Milan	1987
Ripley	New Marion	1987
Ripley	Osgood	1987
Ripley	Sunman	1987
Ripley	Versailles	1987
Rush	Arlington	1851
Rush	Arlington	1868
Rush	Arlington	1885
Rush	Arlington	1902
Rush	—	1919
Rush	Rushville	1987
Saint Joseph	Mishawaka	1885
Saint Joseph	South Bend	1902
Saint Joseph	Walkerton	1902
Saint Joseph	South Bend	1919
Saint Joseph	Granger	1987
Scott	—	1851

County	Town	Year
Scott	—	1868
Scott	Scottsburg	1885
Scott	—	1889
Scott	Lexington	1902
Scott	Scottsburg	1902
Scott	—	1906
Scott	Austin	1919
Scott	—	1970
Scott	Austin	1987
Scott	Blocher	1987
Scott	Leota	1987
Scott	Lexington	1987
Scott	Scottsburg	1987
Scott	Vienna	1987
Shelby	—	1885
Shelby	Flat Rock	1902
Shelby	Shelbyville	1902
Shelby	Flat Rock	1987
Shelby	Shelbyville	1987
Spencer	—	1885
Spencer	—	1898
Spencer	Gentryville	1902
Spencer	Gentryville	1902
Spencer	Huff	1902
Spencer	Lincoln City	1902
Spencer	—	1963
Spencer	—	1976
Spencer	Fulda	1987
Spencer	Gentryville	1987
Starke	English Lake	1885
Starke	Knox	1902
Steuben	Angola	1851
Steuben	Angola	1868
Steuben	Angola	1885
Steuben	Orland	1885
Steuben	Helmer	1902
Steuben	Salem Center	1902
Steuben	York	1902
Steuben	Hamilton	1906
Steuben	—	1932
Steuben	Angola	1987
Steuben	Ashley	1987
Steuben	Hamilton	1987
Steuben	Metz	1987
Sullivan	—	1842
Sullivan	—	1859
Sullivan	Pleasantville	1885
Sullivan	Sullivan	1885
Sullivan	—	1889
Sullivan	Eagle	1898
Sullivan	Sullivan	1898
Sullivan	Alumcave	1902
Sullivan	Jackson Hill	1902
Sullivan	Pleasantville	1902
Sullivan	Sullivan	1902
Sullivan	Dugger	1906
Sullivan	Sullivan	1906

County	Town	Year
Sullivan	Sullivan	1911
Sullivan	Shelburn	1937
Sullivan	—	1963
Sullivan	—	1970
Sullivan	—	1976
Sullivan	Dugger	1987
Sullivan	Hymera	1987
Switzerland	Allensville	1868
Switzerland	Vevay	1885
Switzerland	—	1889
Switzerland	Allensville	1902
Switzerland	Moorefield	1902
Switzerland	Vineyard	1902
Switzerland	Rising Sun	1987
Tippecanoe	—	1834
Tippecanoe	—	1851
Tippecanoe	—	1868
Tippecanoe	Stockwell	1885
Tippecanoe	—	1889
Tippecanoe	Lafayette	1898
Tippecanoe	Lafayette	1902
Tippecanoe	Shadeland	1902
Tippecanoe	Stockwell	1902
Tippecanoe	—	1906
Tippecanoe	Lafayette	1923
Tippecanoe	Lafayette	1936
Tippecanoe	Lafayette	1940
Tippecanoe	—	1970
Tippecanoe	—	1987
Tipton	Windfall	1885
Tipton	Normanda	1902
Union	Clifton	1834
Union	Clifton	1851
Union	Clifton	1868
Union	Clifton	1885
Union	Liberty	1885
Union	Clifton	1902
Union	Liberty	1902
Union	Liberty	1987
Vanderburgh	Inglefield	1868
Vanderburgh	Evansville	1885
Vanderburgh	Inglefield	1885
Vanderburgh	Inglefield	1889
Vanderburgh	Armstrong	1898
Vanderburgh	Evansville	1898
Vanderburgh	Zipp	1898
Vanderburgh	Armstrong	1902
Vanderburgh	Evansville	1902
Vanderburgh	Inglefield	1902
Vanderburgh	Kasson	1902
Vanderburgh	Evansville	1924
Vanderburgh	—	1963
Vanderburgh	—	1970
Vanderburgh	—	1976
Vanderburgh	—	1987
Vermillion	Hillsdale	1868
Vermillion	Clinton	1885

County	Town	Year
Vermillion	Hillsdale	1885
Vermillion	Clinton	1902
Vermillion	Hillsdale	1902
Vermillion	Newport	1902
Vermillion	Cayuga	1987
Vermillion	Clinton	1987
Vermillion	Dana	1987
Vermillion	Hillsdale	1987
Vermillion	Newport	1987
Vermillion	Perrysville	1987
Vigo	Terre Haute	1851
Vigo	—	1868
Vigo	—	1885
Vigo	—	1889
Vigo	—	1898
Vigo	Fontanet	1902
Vigo	Lewis	1902
Vigo	Prairieton	1902
Vigo	Sandford	1902
Vigo	Terre Haute	1902
Vigo	Terre Haute	1919
Vigo	—	1970
Vigo	Terre Haute	1987
Wabash	Richvalley	1885
Wabash	—	1902
Warren	Pine Village	1885
Warren	State Line	1885
Warren	Pine Village	1902
Warren	State Line	1902
Warren	West Lebanon	1902
Warren	—	1970
Warren	—	1987
Warrick	Elberfeld	1885
Warrick	—	1889
Warrick	Chandler	1898
Warrick	Newburgh	1898
Warrick	Boonville	1902
Warrick	Chandler	1902
Warrick	Elberfeld	1902
Warrick	Paradise	1902
Warrick	Stanley	1902
Warrick	—	1906
Warrick	Elberfeld	1936
Warrick	—	1963
Warrick	—	1970
Warrick	—	1976
Warrick	Selvin	1987
Washington	Salem	1851
Washington	Salem	1868
Washington	Salem	1885
Washington	Salem	1885
Washington	—	1889
Washington	Bartle	1902
Washington	Salem	1902
Washington	—	1906
Washington	—	1970
Washington	—	1974

County	Town	Year
Washington	Campbellsburg	1987
Washington	Fredericksburg	1987
Washington	Hardinsburg	1987
Washington	Martinsburg	1987
Washington	New Pekin	1987
Washington	Salem	1987
Wayne	Economy	1868
Wayne	Richmond	1868
Wayne	Economy	1885
Wayne	Economy	1902
Wayne	Jacksonburgh	1902
Wayne	Richmond	1902
Wayne	Richmond	1906
Wayne	Richmond	1919
Wayne	Hagerstown	1936
Wayne	Richmond	1936
Wayne	—	1970
Wayne	Richmond	1987
Wells	Bluffton	1885
Wells	Bluffton	1898
Wells	Bluffton	1902
White	Wolcott	1868
White	Monticello	1885
White	Wolcott	1885
White	Monticello	1902
Whitley	—	1885
Whitley	South Whitley	1902
Whitley	Columbia City	1987

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Observations on Periodical Cicadas (Brood X) in Indiana in 1987 (Homoptera: Cicadidae)

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Abstract

Periodical cicadas of Brood X (*Magicicada septendecim, cassini*, and *septendecula*) emerged in large numbers in three large areas in the eastern United States. The nympal emergence holes and turrets similar to those of crawfish were observed in early May, and emergence of adults occurred shortly afterwards in forested areas east of Bloomington in Indiana. The first adults emerged on the Indiana University campus on the night of May 12-13. The largest emergence occurred between May 17 through the 29th. The last observed emergences occurred during the morning of June 4. The response to a computerized solicitation of records from Indiana and Ohio county agricultural agents made by the junior author indicated a very extensive emergence in those states. Records from the northern tiers of counties in both states are the first since the early part of the century. Cicadas (presumably of Brood XIV) in Brown County in Indiana emerged at the same general time as those from adjacent counties which suggests that the large broods have absorbed smaller ones by some such acceleration of emergence.

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The emergence of periodical cicadas or 17-year locusts of the genus *Magicicada* has been observed in the eastern woodland areas of the United States since 1633 or 1634 when the Pilgrims of Plymouth Colony first encountered them. At this early date the cicadas came out of the ground in the spring and made the woods ring with their "singing" according to Moreton (1). The colonists had never seen such insects, but the Amerindians had, and predicted that pestilent fever would follow. The cicadas "sang", mated, layed their eggs, and died, and as the hot summer wore on the fevers came killing many of the colonists and Indians alike. In the autumn the pestilence abated, but it reappeared the following year. The Pilgrim "flies" or "locusts", however, were not seen again until 1651. They have since appeared as if regulated by clockworks every 17 years down to the last recorded emergence in 1974. Fortunately, the correlation with pestilent fevers has not proven constant.

The emergence of Brood X of the 17-year or periodical cicadas or 17-year locusts occurred in the three large areas in which the cicadas have appeared in previous emergences since 1715. In Indiana the three 17-year species of *Magicicada* occurred in most areas of emergence in about the same ratios observed previously. *Magicicada septendecim* (Linnaeus) was most numerous and *M. cassini* (Fisher) and *M. septendecula* Alexander and Moore were present in smaller numbers. On the Indiana University campus the two smaller species emerged later than *M. septendecim*, Young (2).

For the first time the senior author was able to detect mating aggregations on the Indiana University campus. Choruses of the males occurred at various points on the central wooded campus and in the vicinity of some outlying wooded areas. Very large choruses were reported and observed in Greene and other counties in Southern Indiana. The accompanying map summarizes the records for Indiana. Large black circles indicated a widespread and heavy emergence over most of a county. Smaller circles indicate light or spotty emergence. In nearly all cases examination of ovoposition scars, nymphs, or adults indicate that *M. septendecim* was the more numerous. The absence of records from central Indiana probably reflects the early and heavy clearing of these areas for agriculture. The cicadas have probably not had time to spread back into these areas to invade the wooded areas formerly cleared. Observations in several places makes it unlikely that the cicadas followed up stream gallery forests. Even moderate flooding seems to be enough to eliminate them although *M. cassini* shows some preference for lower situations.

It is still uncertain if the cicadas (like songbirds) have profited from the edge effects produced by forest clearing. They do emerge earlier and possibly in larger numbers along the edges of woods, but close observation on closed forest situations may underestimate the number of emergents. That is, in the mature beech-maple or other mature forests the cicadas climb up rapidly into the taller trees, and the estimation of emergence by counting exuviae may result in underestimation. On the other hand, the emergences in 1987 seemed equal to or greater than the emergences in 1970 and 1953 observed by the senior author.

Daily observations were made on the Indiana University Campus on eight areas differing somewhat in vegetation, slope, and drainage. These areas are roughly about 10 by 20 meters in which all adults and exuviae that could be found were collected. Emergence began nearly a week earlier than in 1970 and continued 1 to 3 days longer. Statements by previous workers suggesting a remarkable coordination of the emergences was not observable. Time and place of emergence was obviously influenced by isolation and ambient temperatures. Emergences early along the edges of woodland fragments was noted as in 1970, but as stated elsewhere it is not certain that this indicates a true edge effect. As the accompanying table shows any tendency for all cicadas, even in a restricted area, to emerge synchrously is masked by the local differences. [See Young (2)]

Areas may be described briefly as follows:

Area 1. A fragment of mature forest, predominantly beech and maple, shaded by buildings on the east and west (Jordan and Myers halls). Essentially flat, well drained. This and all areas except 7 and 8 not compacted by crosswalks or other traffic. Tree shade heavy.

Area 2. Cleared area (lawn) shaded on west by low temporary building. Well drained. Tree shade light. Emergence primarily around large beech trees.

Area 3. Part of mature forest fragment on gentle north-facing slope, predominatly mature beech-maple with understory of pawpaw and a few other shrubs and vines. Well drained. Emergence heaviest along open south edge. Tree shade moderate.

Area 4. Area of mixed forest on either side of small temporary stream. Drainage in part poor. Tree shade heavy. Emergence heaviest along open south edge.

Area 5. Area within mature beech-maple forest fragment on gentle south-facing slope (central quadrangle of campus). Understory of pawpaw and other shrubs. Drainage good. Tree shade moderate to heavy.

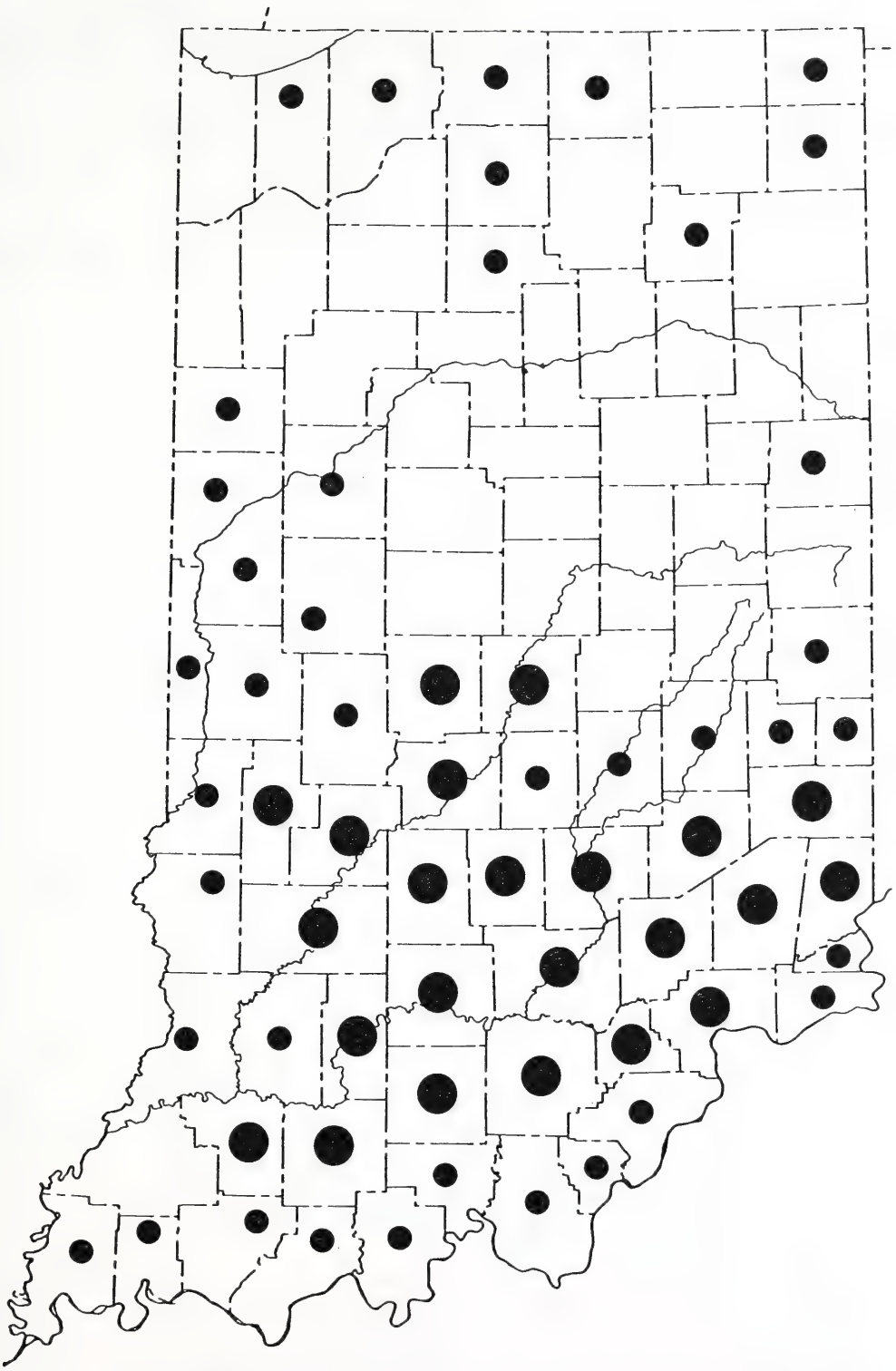

FIGURE 1. Map of Indiana showing counties and records of emergence of 17-year cicadas of Brood X in 1987. Larger circles indicate heavy and widespread emergences. Smaller circles indicate local or limited emergence. Three species of *Magicicada* were present in the emergences in southern Indiana. Map based on reports of county agricultural agents solicited by the junior author and personal observations.

Area 6. Area at edge of mature beech-maple forest fragment on other side of a temporary stream (central quadrangle of campus). Understory of pawpaw and other shrubs. Drainage generally good. Tree shade moderate to heavy. Emergence heaviest along open eastern edge.

Area 7. Lawn area immediately east of 6. Area compacted by mowing and foot traffic. Drainage good. Tree shade light. Emergence mainly around large beech trees.

Area 8. (As indicated on table, had to be replaced by area 8A which was the closest area available, a grassy area near Mitchell Hall. Emergence here was heavier than the adult count indicates, but was primarily of *M. septendecim*.

BROODS OF THE PERIODICAL CICADAS
(After Marlatt, 1907)

17-year broods

Brood	Early Dates	Distribution	Expected Next
I	1808	D.C., Ill., *Ind.*, Kan., Ky., Md., N.C., Pa., Va., W. Va. (Last Ind. record—1859!)	1995
II	1724	Conn., D.C., *Ind.*, Md., Mich., N.J., N.Y., N.C., Pa., Va., W. Va. (Last Ind. record—1911!)	1996
III	1844	Ill., Iowa, Mo., Neb., Ohio, W. Va.	1997
IV	1845	Ark., Okla., Iowa, Kans., Mo., Neb., Tx.	1998
V	1795	Ohio, Pa., Va., W. Va.	1999
VI	1847	*Ind.*, Del., D.C., Ga., Ill., Ky., Md., Mich., N.J., N.Y., N.C., Ohio, Pa., S.C., Tenn., Va., W. Va., Wisc. (Widely distributed, but scattered.) (Last recorded from Indiana in 1983.)	2000
VII	1797	N.Y., Pa., W. Va.	2001
VIII	1815	Ill., Md., Mass., N.J., N.Y., N.C., Ohio, Pa., S.C., W. Va., ? Ind. (1934)	2002
IX	1833	N.C., Ohio, Va., W. Va.	2003
X	1715	Ala., D.C., Del., Ga., Ill., *Ind.*, Iowa, Ky., Md., Mass., Mich., N.J., N.Y., N.C., Ohio, Pa., Tenn., Vt., Va., W. Va., Wisc. (Largest of the 17-year broods.) (Last Ind. Record—1987.)	2004
XI	1767	Conn., Mass., R.I.	1988
XII	1853	Ill., *Ind.*, Md., Ohio, W. Va. (Last Ind. Record—1938 in Orange County.)	1989
XIII	1854	Ill., *Ind.*, Iowa, Mich., Pa., Wisc., Ky., Md., Va., W. Va. (Last Ind. Record—1956.)	1990
XIV	1634	D.C., Ga., Ill., *Ind.*, Ky., Md., Mass., N.J., N.Y., N.C., Ohio, Pa., Tenn., Va., W. Va., Ala.	1991
XV	1839	N.J., N.Y., N.C.	1992
XVI	1857	Ark., Iowa?, Neb.	1993
XVII	1841	Ga., Iowa, Mo., N.Y., Va.	1994

Table 1. Records for Emergence of *Magicicada* species Adults Listed in Two Day Intervals for selected areas on the Indiana University Campus, Bloomington, Indiana

Area	Species	Dates of Emergence for May-June 1987												Total
		13-14	15-16	17-18	19-20	21-22	23-24	25-26	27-28	29-30	31-1	2-3	4-5	
1	S	0	17	46	53	45	12	62	34	7	0	0	1	277
	Sc	0	8	23	30	17	29	35	20	5	0	0	0	169
	C	0	1	17	13	23	4	26	4	1	0	0	0	89
2	S	1	20	1	33	2	0	5	0	2	0	0	0	84
	Sc	1	22	22	3	12	0	1	0	3	0	0	0	163
	C	0	0	1	14	0	0	1	1	0	0	0	0	17
3	S	0	4	4	3	4	20	3	19	4	0	0	0	61
	Sc	1	14	1	7	6	11	0	9	4	1	0	0	54
	C	0	2	7	6	6	9	1	0	1	1	0	0	33
4	S	0	0	5	13	21	18	36	0	1	4	11	0	99
	Sc	0	0	0	0	2	48	0	0	0	1	10	0	61
	C	0	0	1	5	3	23	10	7	2	6	0	0	57
5	S	0	0	3	6	21	95	43	25	10	5	0	0	211
	Sc	0	0	0	0	2	8	11	7	0	0	0	0	28
	C	0	0	0	0	3	29	3	34	1	5	0	0	75
6	S	0	0	4	14	14	17	2	20	1	0	0	0	72
	Sc	0	0	0	0	0	1	0	2	0	0	2	0	5
	C	0	0	0	4	8	5	0	3	1	0	0	0	21
7	S	0	0	11	5	3	3	4	0	7	1	0	0	34
	Sc	0	0	0	0	0	0	0	0	0	0	0	0	0
	C	0	0	0	0	4	2	3	0	1	0	0	0	10
8A**	S	0	0	3	1	0	0	0	0	0	0	0	0	4
	Sc	0	0	0	0	0	0	0	0	0	0	0	0	0
	C	0	0	0	0	0	0	0	0	0	0	0	0	0
													Total	1622

*S = Magicicada septendecim (Linnaeus)
Sc = Magicicada septendecula Alexander & Moore
C = Magicicada cassini (Fisher)
**Area 8A is a substitute for Area 8 of 1970 made necessary by destruction of former Area 8.

BROODS OF THE PERIODICAL CICADAS
13-year broods

Brood	Early Dates	Distribution	Expected Next
XVIII	1818	Ala., N.C., Ga., S.C., Tenn.	1997
XIX	1803	Ala., Ark., Ga., Ill., Okla., *Ind.*, Iowa, Ky., La., Miss., Mo., N.C., S.C., Tenn., Texas, Va., (Largest of the 13-year broods.)	1998
XX	1843	Ga., N.C.?, Va.?	1999
XXI	1844	Ala., Fla., Miss., Tenn.	2000
XXII	1806	La., Miss.	1988
XXIII	1820	Ala., Ark., Ga., Ill., *Ind.*, Ky., La., Miss., Mo., Ohio, Tenn., (Next Largest of 13-year broods.) (Last Indiana record—abundant in 1976.)	1989
XXIV	1873	Ark., Ill., La., Miss., Mo.	1990
XXV	none	no brood	—
XXVI	1849?	La., Miss., Texas?	1992
XXVII	1902	Miss.	1993
XXVIII	none	no brood	—
XXIX	1839?	Ga., Okla., S.C.	1995
XXX	1892	La., Mo.	1996

In Brown County, Indiana Brood XIV apparently emerged at the same time as Brood X in adjacent counties and in some parts of Brown County. Some emergence was also observed in Crawford and Harrison counties where Brood XIV was abundant in 1974 [Young (3)]. At that time there was some indication of restriction of this brood. Records for several southern Indiana counties could not be confirmed. It is possible that the emergence of Brood X from neighboring areas in some manner triggered emergence of Brood XIV. This suggests that a four-year acceleration of a brood might be frequent enough to influence the abundance of another brood. Thus Brood X and Brood XIV might owe their greater abundance and distribution to having absorbed some other broods. (This hypothesis is explored further in an accompanying paper by the junior author.)

Although Lloyd and Dybas (4) and others consider the four-year interval as important in brood emergence this is not positively proven and shorter periods of acceleration are certainly possible if not frequent.

Again during the 1987 emergence no real overlapping could be observed with areas where Brood XXIII emerged in southern Indiana counties in 1976 (Young, 5).

The construction by nymphs of "turrets" above the surface at the end of their burrows has been puzzling to workers in the past. It does not seem to be necessary for protection against flooding since the turrets are constructed in well drained as well as poorly drained situations. It has been observed during several emergences that moles are particularly active in the surface layer of the soil at the time the cicada nymphs are burrowing up to the surface. The relatively small turrets may offer a safe retreat out of the unusual hunting zone of the moles.

Acknowledgements

We wish to acknowledge the assistance of Mary Ann Gudeman who carried out observations on the Indiana University campus and mounted and labelled the numerous voucher specimens.

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ABSTRACTS

An Evaluation of Botanical Air Purification as an Indoor Formaldehyde Mitigation Measure. Thad Godish. Indoor Air Quality Research Laboratory, Ball State University, Muncie, Indiana 47306.—The ability of spider plants to remove formaldehyde from contaminated air was investigated under controlled laboratory chamber conditions. Formaldehyde levels were observed to be quantitatively reduced by the presence of spider plants in a chamber environment in which formaldehyde was continuously produced from particleboard materials. The greatest degree of formaldehyde reduction occurred when plants had been totally defoliated. These results do not support conclusions published previously that spider plants remove formaldehyde from contaminated air. These studies suggest that formaldehyde removal is effected by the soil environment rather than the foliage of spider plants.

The Impact of Residential Wood Combustion on Indoor Particulate Matter Levels. Randy Kirk and Thad Godish. Hayes Regional Arboretum, 801 Elks Road, Richmond, Indiana 47374, Indoor Air Quality Research Laboratory, Ball State University, Muncie, Indiana 47306.—Serious environmental costs have been associated with residential wood burning. This study concentrates on indoor air pollution, specifically particulate matter, as a result of wood burning in the home. Three private residences located at Hayes Regional Arboretum in Richmond, Indiana equipped with identical wood burning stoves and using a controlled wood supply, were monitored for a 48-day period, collected particulate matter, measuring wood weight burned, noting stove temperatures and timing open burn periods during refueling. Suspended particulate matter was captured on paper tape samplers and analyzed for changes in optical density to determine the coefficient of haze. The primary objective was to determine the significance of burn temperature and open burn time on particulate matter levels in the 3 houses.

A Self-extinguishing Safer Cigarette. Paul D. LaHue, 1137 Linden Drive, Bloomington, Indiana, 47401.—Research to develop a delivery system to treat cigarette tobacco with a safe inorganic fire-retardent has produced a self-extinguishing cigarette. The process and invention thereof pertains generally to the manufacturing of cigarettes by presently used cigarette-making machines. Burning cigarettes can and do start fires. Inhaling of cigarette smoke is detrimental to the health of smokers and non-smokers

alike. A delivery system attached to the cigarette-maker treats the tobacco particles, preventing a cigarette from burning beyond a determined butt length. The delivery system and treatment is controlled by the cigarette-maker's computer and synchronized to the speed of the cigarette production. This research can well result in a cigarette that will greatly reduce the fire hazards and also the health hazards of cigarette smoking.

Rain and Dew as Urban Acid Deposition Scavengers. Robert A. Pribush, Bradley H. Carter, Jennifer L. Waugh and Kimberly A. Bridges, Department of Chemistry and the Holcomb Research Institute, Butler University, Indianapolis, Indiana 46208.—For the past two years, wet and dry deposition samples have been collected at the Butler University Environmental Preserve (BUEP) in Indianapolis and the samples analyzed according to protocols adapted from the National Atmospheric Deposition Program (NADP). Monthly, seasonal, and annual comparisons of data from the BUEP, an urban site, and the NADP, a network of exclusively rural sites are being made to ascertain the possibility of using rainfall and/or dry-deposition data as diagnostic tools for local influences on air quality.

The use of dew as a scavenging agent will also be discussed. Preliminary analysis of recent dew collections show that dew is significantly different from rainfall or fog collected at the BUEP in the concentrations and types of major ions routinely present in these samples, particularly with respect to those species which have an influence on deposition acidity. Comparisons of dew data from the BUEP with data from studies in Pennsylvania and Michigan indicate that dew composition is considerably more site specific than rainfall composition.

Detection and Determination of Chemical Constituents in Water. Joseph R. Siefker and Stephen K. Moore, Department of Chemistry, Indiana State University, Terre Haute, Indiana 47809.—Chemical assays were made on selected samples of water taken from the Wabash River at Terre Haute and the tap in a chemistry laboratory at Indiana State University. Appropriate procedures were used to determine calcium, chloride, copper, dissolved oxygen, hardness, pH, phosphate, solids, specific conductance, sulfate, and zinc.

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An Assessment of Water Quality on Little and Big Duck Creeks Near Elwood, Indiana

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Introduction

This research was conducted in 1978-79 to determine the effects of discharges and surface runoff from Elwood, Indiana, on the water quality of Big and Little Duck Creeks. These results were then compared to findings of 1938. The major contributor of pollutants was wastewater from homes, industry, and an overworked sewage treatment plant. This problem has been documented by the Indiana State Board of Health, the Anderson Water Pollution Control Department, and the Madison County Health Department (2,5,6).

The first extensive survey of Big and Little Duck Creeks was conducted by the Indiana State Board of Health from 1928 to 1929 (5). At that time, the stream was used as an open sewer. This resulted not only in unpleasant appearances and offensive odors, but also created a potential health hazard.

No follow-up study has been conducted since 1938 to compare the water quality of Duck Creek above and below Elwood. It was, consequently, of interest to compare water quality changes over a 40 year period.

Description of Study Sites

Big Duck Creek drains a 36 square mile area north of Elwood (Fig. 1) The stream flows in a southwesterly direction through Elwood toward the west fork of White River. The discharge of the creek is generally low, but increases considerably below the sewage treatment plant (4).

To determine the effects of Elwood on the water quality, three stations along Big Duck Creek and two stations on its tributary, Little Duck Creek, were established.

Materials and Methods

Samples were collected during the middle of the week in the late afternoon between June 13, 1978, to January 12, 1979. Samples were analyzed for dissolved oxygen (DO), biochemical oxygen demand (BOD), suspended solids, ammonia, total coliform bacteria and hydrogen ion concentrations (pH). Sampling and analyzing was conducted as described in Standard Methods (1).

All data collected in this and the previous study were statistically analyzed. Differences in sample means for each of the six parameters measured, based on comparisons of values at each sampling location in the 1978-79 study, were determined by the use of a completely Randomized Design Analysis of Scheffe' Test for Multiple Comparisons. The student's t-test was used to determine differences of sampling means of environmental parameters from the 1938 to 1978-79 study. A probability level of 0.05 was accepted as significant.

Results

Means and standard deviations for environmental parameters for Big and Little Duck Creeks for studies conducted in 1979 and 1937 are summarized in Table 1.

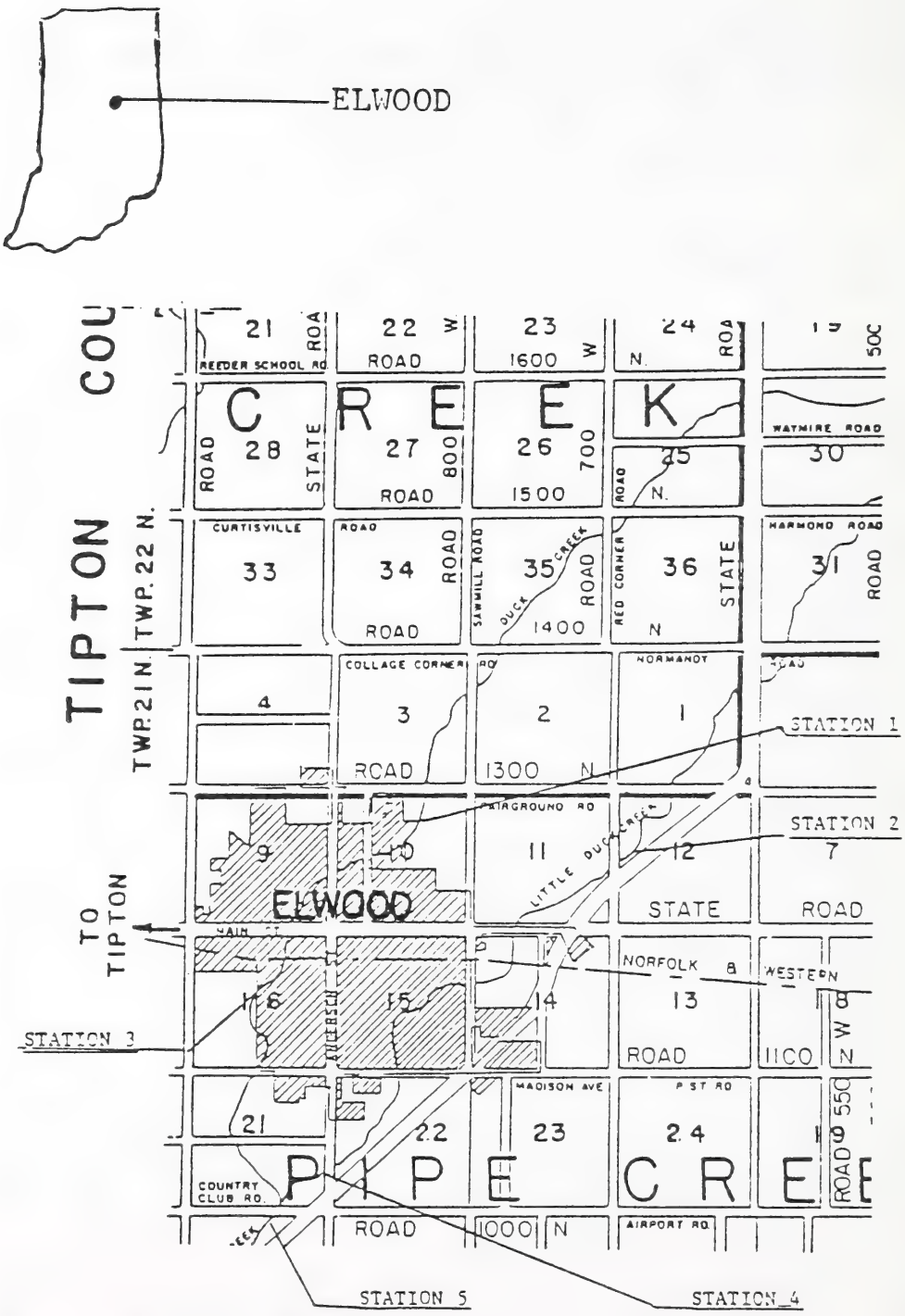

FIGURE 1. Location of Study Sites.

The mean DO concentrations at Station 1 of Big Duck Creek were higher (9.1 mg/1) than the remaining reaches of Big Duck Creek. A similar trend was observed for Station 2 on Little Duck Creek.

Aeration of the still, shallow portions of the streams, photosynthesis of aquatic plants and low concentrations of BOD may be responsible for the high DO in the upstream areas (Table 1).

TABLE 1

Means and Standard Deviations (SD) for Dissolved Oxygen Concentrations (DO) Hydrogen Ion Concentrations (pH), Biochemical Oxygen Demand (BOD), Suspended Solids (SS), Total Coliform Bacteria (TCB-colonies X10^3/100ml), and Ammonia Concentration (NH_3) of Little and Big Duck Creek from June 13, 1978, to January 12, 1979. All mean values are expressed as mg/1 with the exception of pH and TCB.

	Stations				
	1	2	3	4	5
Parameter			1978-79		
DO	9.1 ± 3.3	7.04 ± 4.2	5.1 ± 4.3	6.9 ± 3.1	5.4 ± 2.9
pH	8.2 ± .20	8.0 ± .21	7.8 ± .30	7.9 ± .30	7.9 ± .14
BOD	2.5 ± 1.9	5.6 ± 4.7	5.8 ± 2.9	4.2 ± 2.7	7.3 ± 2.8
SS	5.8 ± 4.3	3.8 ± 3.1	10.6 ± 10.4	6.8 ± 5.2	9.4 ± 9.5
TCB	13.1 ±18.2	38.8 ±41.5	310.4 ±272.8	17.7 ±23.5	20.8 ±22.0
NH_3	0.81± 0.66	0.29± 0.21	0.37± 0.20	0.20± 0.21	0.53± 0.65

Station 3 had the lowest mean DO concentration (5.1 mg/1). This oxygen level was probably reduced by the higher BOD which was observed at this sampling location. Wastewater storm overflow, urban runoff and untreated household wastewater discharges were the most probable sources of oxygen-depleting wastes affecting this segment of Big Duck Creek.

The DO concentrations at Stations 2 (7.0 mg/1) and 4 (6.9 mg/1) located on Little Duck Creek were still high. This was surprising since malfunctioning household on-site sewage disposal systems were observed discharging into Little Duck Creek.

The DO levels decreased at the downstream Stations 3 and 5. This may have been caused by agricultural runoff and the introduction of oxygen-demanding waste from the town.

Results from this study show that the mean DO concentration in the upstream region of Station 1 was 4 mg/1 higher in 1978-79 than in 1937. The 1938 report by the Indiana State Board of Health indicates that this part of Big Duck Creek

TABLE 2

Means and Standard Deviations for Dissolved Oxygen Concentrations (DO), Biochemical Oxygen Demand (BOD), Suspended Solids (SS) and Total Coliform Bacteria (TCB-colonies X10^3/100ml) of Little and Big Duck Creek from August 29, 1937, to September 23, 1937. All mean values are expressed as mg/1 with the exception of TCB.

	Stations				
	1	2	3	4	5
Parameter			1937		
DO	5.0 ± 3.8	7.1 ± 3.6	0.4 ± 0.9	7.3 ±3.2	0.06±0.2
BOD	18.9 ±25.4	2.8 ± 1.7	354.7 ± 20.13	40.2 ±2.8	—
SS	54.2 ±31.6	110.5 ±47.4	185.6 ± 144.3	40.07±7.8	—
TCB	3.90±45.842	.055± .052	1835.70±3485.00	.30±0.40	1000.00±0000

experienced low flow such that the stream often reached pool stage (5). It was also indicated that mud in the channel was occasionally stirred up in a hog wallow upstream. This condition was improved by 1979 when the hog wallow was eliminated and the stream flow was increased due to changes in agricultural drainage.

Station 2 was above all known sources of pollution in 1937, except for agricultural runoff and the DO levels were similar in both periods.

In the downstream portion from Stations 1 and 2, the DO levels were noticeably lower during the 1937 investigation. The inner city mean DO level in 1937 was 0.4 mg/1 as compared to 5.1 mg/1 in 1978-79. A similar difference occurred at Station location 5 below Elwood, where the mean DO level in 1937 was a barely detectable 0.06 mg/1 while in 1978-79 it was 5.40 mg/1. The most obvious reason for the improvement in DO may be because Big Duck Creek is no longer being used as an open sewer as it was 40 years ago.

Station 1 had the lowest mean BOD (2.5 mg/1) of all 5 Stations. This may be due to this station being above major sources of organic pollution. At Station 2, the mean BOD was considerably higher (5.6 mg/1) and may have resulted from the application of animal manure on the fields near the stream.

Station 3 had a higher BOD (5.8 mg/1) than the upstream water of Big Duck Creek. As was mentioned before, faulty household sewerage systems and a municipal sewer overflow probably provided the organic waste.

Station 5 had the highest BOD level (7.3 mg/1). This was most likely due to the inner city wastewater discharges, as well as effluent from the Elwood sewage treatment plant.

The 1978-79 study showed a 16 mg/1 decrease in BOD at Station location 1. This decrease was probably due to the elimination of the hog wallow. BOD levels were significantly lower in 1978-79 at Station 2 as compared to the 1938 study, even though land use had not changed much in that time.

The mean BOD concentration at Station 3 was 354 mg/1 in 1937 compared to only 5.8 mg/1 in 1978-79, because of effluents from several tomato canneries, as well as raw domestic sewage at that time. These wastes are no longer a part of the organic load on this portion of the stream.

BOD levels at Station 4 in 1937 were significantly higher (40 mg/1) than those measured in 1978-79 (4.2 mg/1). Apparently, Little Duck Creek was used to carry away domestic waste in 1937. Suspended solids levels (SS) were low at the upstream stations. The highest levels were determined at Station 3 in 1978-79. Apparently, addition of city effluent to the already existing load of soil particles increased the SS concentration.

Suspended solids levels in 1937 were significantly higher as compared to 1978-79 at all stations. The most probable reasons for these differences include higher levels of erosion in 1937 and discharges of raw sewage together with other organic wastes from a hog operation located north of Elwood abutting Big Duck Creek.

Total coliform bacterial (TCB) counts were significantly higher at Station 3 (310 x 10^3/100 ml) in 1978-79 than at any other station. This sampling location was affected by a wastewater overflow immediately upstream and also received wastewater from household sewer systems. At Station 5 total coliform bacterial counts were only a fraction (20.8^3 x 10/100 ml) of the concentration level found at Station 3. The reason for this difference may be due to chlorination of wastewater at the sewage treatment plant.

Total coliform bacterial counts were lower at Stations 2 and 4 in 1937 than in 1979. This was apparently due to limited development along Little Duck Creek at that time. However, the bacterial count was ten times higher in 1937 than in 1979 at Station 3. This was probably due to untreated industrial and household waste.

Mean ammonia levels did not increase significantly within the city. Station 3 exhibited a mean level of 0.27 mg/1. Station 4 had a similar level of 0.20 mg/1. The relatively low concentrations of ammonia at these stations were probably due to the limited time available for ammonia production from organic matter.

Station 5 on Big Duck Creek exhibited a mean ammonia concentration of 0.53 mg/1. This higher value was probably due to ammonia in the effluents from the sewage treatment plant.

No ammonia determinations were made in 1937, consequently, no comparison could be made. Generally, the pH levels varied little from station to station.

Conclusion

When the upstream and downstream stations were compared, a definite decrease in water quality was noted. The DO concentrations decreased as the water flowed through the city. The BOD increased in the downstream area because of increased organic loadings.

Elwood increased the SS level of Big Duck Creek. Agricultural and urban runoff together with untreated sewage significantly increased SS within the inner city region. Dilution by treated sewage, as well as, cleaner water from Little Duck Creek helped to reduce the suspended solids level in the downstream location.

Relatively high ammonia concentrations were observed in the upstream portions of the creeks. This was apparently related to farm practices. The higher readings of ammonia were noted after animal manure was spread on the fields along the stream. The downstream levels were also high. This was probably due to ammonia in the effluents from the sewage treatment plant. In contrast, only small changes in the hydrogen ion concentration were observed along the creek.

Total coliform bacteria increased in the center of town because of a sewer bypass into the creek. Below Elwood the concentration of bacteria decreased due to dilution with disinfected effluents from the sewage treatment plant.

Except for a noticeable increase in BOD and ammonia, the Elwood sewage treatment plant effluent together with water from Little Duck Creek appeared to improve Big Duck Creek's condition as it left the city to join White River.

Significant differences between parameters in 1937 and 1978-79 were observed. Results of this study indicate a significant improvement in water quality of Big Duck Creek since 1937. This included significant decreases in biochemical oxygen demand, suspended solids, total coliform bacteria and increases in dissolved oxygen. This was probably due to the concentration of Elwood's wastewater collection system and sewage treatment plant in the 1940's. However, there was still degradation of water quality within the city due to untreated wastewater discharges. Consequently, emphasis should be placed on the inner city problem.

Acknowledgments

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ABSTRACTS

Remote Sensing with Fiber Optics in Oil and Soil Exploration. TORSTEN ALVAGER, Department of Physics, Indiana State University, Terre Haute, Indiana 47809; ASHOK PURI, Department of Physics, University of New Orleans, New Orleans, Louisiana and RAMNATH RAYMOND, Department of Physics, Indiana State University, Terre Haute, Indiana 47809.——The recent introduction of fiber optical light guides for remote spectroscopic sensing has opened a new era of studies of hostile or otherwise inaccessible environments. The method is also of importance in a variety of other situations such as in studies of solid state systems and systems related to damage and environmental control, as well as in experiments devoted to search for oil and other similar commodities, which is the topic of this investigation.

An instrument especially designed for in situ investigations has recently been built at Indiana State University for this purpose. This makes it possible to study scattering as well as fluorescence from samples of interest. Results with model systems indicate that the method is suitable for field use in oil and soil exploration.

Bedrock Joint and Fracture Patterns in Indiana: A Progress Report. CURTIS H. AULT, Indiana Geological Survey, 611 N. Walnut Grove, Bloomington, Indiana 47405.——Bedrock joints and fractures are ubiquitous where bedrock is exposed in Indiana. Recent studies of the patterns, orientation, and density of jointing in Indiana have revealed a consistent east-northeasterly orientation for major jointing and a corresponding north-northwesterly orientation for secondary orthogonal jointing. There is an apparent counterclockwise shift of about 50° in orientation in the southern tier of counties of southwestern Indiana. Although most of the orientations of the jointing can be attributed to regional lithospheric stress, orientations at some isolated localities are better explained by rock inhomogeneities, settling of reef-flank beds, or topography and structure. A few anomalies are not yet explained. Intriguing applications and potential uses of these data include comparison of directions of bedrock jointing with directions of jointing in compact glacial till as possible indicators of differing genesis; applications to operations of surface and underground mines for limestone, dolomite, and coal; and characterization of fracture permeability in bedrock, particularly where solution channels have occurred along joints.

Distribution and Sources of Iron in the Salem Limestone of South Central Indiana. MARK A. BROWN, DONALD D. CARR, MARGARET V. ENNIS, R. K. LEININGER AND LOUIS V. MILLER. Indiana Geological Survey, Bloomington, Indiana 47405.——The

Mississippian Salem Limestone, the nation's leading dimension limestone, also is suited for other uses, including manufacture of lime, cement, and glass. For lime and cement, iron content is not critical; but for glass, iron should not exceed 0.1%.

Elemental analyses of Salem facies reveal that iron content correlates with insoluble-residue concentration. Gainstone (dimension-stone) facies contain an average of 2% insoluble residue and an iron content less than 0.1%. Micritic and dolomitic (bastard-stone) facies contain 4 and 11% insoluble matter, respectively, and a combined average of 0.6% iron. Depositional environment apparently controlled the accumulation of insoluble residues and, thereby, the distribution of iron. Insoluble materials were winnowed out of high-energy environments but accumulated in the lower energy settings.

Chemical studies show that pyrite is the dominant iron-containing mineral. Reducing conditions necessary for pyrite formation were produced in association with organic matter, a major insoluble component in micritic and dolomitic facies. Illite, a common insoluble material, is a potential but minor source of iron.

Cuttoff Sandstone and Succession of Upper Pennsylvanian Strata in the Wabash Valley. GARRE A. CONNER, Departments of Geology and Biology, Indiana University, Bloomington, Indiana 47405.——D.D. Owen, 1837, described upper Pennsylvanian strata at the Cuttoff on the Wabash River below New Harmony referring to the Cuttoff sandstone; a logial name extensible to contemporary investigations. Sandstone exposures in the lower Wabash Valley are petrologically similar occuring in cyclic fluviatile and deltaic sequences representing members from St. Wendel to Merom sandstones. C.E. Wier, 1955, described exposures in Posey, Gibson, and Knox Counties omitting the Cuttoff, but imparted the inference that the Cuttoff sandstone may be equivalent with the St. Wendel or the Merom. G.A. Conner, 1987, identified *Neuropteris* variation in the Raben Branch coal flora related to Medullosan seed fern expansion associated with the Westphalian-Stephanian Euramerican time boundary. Further investigation of floras in the Cuttoff sandstone reveal variations not present below the St. Wendel sandstone. Analysis of *Neuropteris* variations in shales associated with Merom sandstone may provide a basis for resolving equivalency with the Cuttoff sandstone.

Early Pennsylvanian Landscape in Western Indiana. JOHN B. DROSTE AND STANLEY J. KELLER; (JBD) Department of Geology, Indiana University, Bloomington, Indiana 47405 and (SJK) Indiana Geological Survey, 911 North Cottage Grove, Bloomington, Indiana 47405.——About 325 million years ago, in very early Pennsylvanian time, the place now called Indiana was subject to subaerial erosion. Data from approximately 20,000 wells provide the evidence to reconstruct the drainage pattern for western Indiana and to show the relationship of six ancient physiographic regions to the eroded outcropping Mississippian bedrock. The stream courses of four primary rivers and of several dozen major rivers and major creeks have been mapped. Each physiographic region was the surface locus of specific stratigraphic units. From north to south a distincitve region is associated with each of the ancient outcrop areas of the Borden Group, the Sanders and Blue River Groups, the West Baden Group, the Stephensport Group, the Tar Springs Formation through the Menard Limestone, and the Palestine Sandstone through the Grove Church Shale. This entire area was covered by renewed sedimentation during Early Pennsylvanian time.

Southwestern Indiana's Abandoned Coal Mine Problems. JULIE ELBERT, University of Southern Mississippi, Hattiesburg, Mississippi 39406-5051 and LEE GUERNSEY, Indiana State University, Terre Haute, Indiana 47809.——Coal has been and continues to be an important economic activity in the state of Indiana. The extraction of a resource is not without an impact on man and the environment. Prior to the Surface

Mining Control and Reclamation Act of 1977 lands affected by coal mining were not required by law to be reclaimed. The lands mined prior to 1977 are termed "Abandoned Coal Mine Lands." The National Update on Abandoned Mine Lands was charged with inventorying and prioritizing these problems. The general results of this Update are presented here for Indiana. There are over 1,070 danger problems and greater than 5,577 acres are affected in seventeen southwestern Indiana counties.

"Weatherwatchers" and You: Viewer Responses to Television Severe Weather Warnings. WILLIAM A. GUSTIN, Department of Geography and Geology, Indiana State University, Terre Haute, Indiana 47809.——Television severe weather warnings are the primary means of notifying the public of dangerous weather conditions. However, there are increasing indications of shifting attitudes of the public to the severe weather warning symbols displayed on television. In this presentation, comparisons are made between a study of viewer responses to severe weather warnings, which was published in 1982, and a study conducted in the spring of 1987 by the author of this presentation. Both of these studies were centered on the WTHI-TV 10 viewing area in west-central Indiana and east-central Illinois. Also, the same set of 12 viewer response questions were used in each study. The main difference between these 2 studies was the way the surveys were conducted. The first survey was distributed as a random sample, while the latter one was distributed through the WTHI-TV 10 "weatherwatcher" network. This also allowed for a comparison of the attitudes of weatherwatchers and non-weatherwatchers. WTHI-TV 10 in general and Kevin Orpurt, Weather Director at WTHI-TV 10, in particular, as well as the weatherwatcher network were instrument in the completion of this project.

Distribution of Sulfur Forms in Indiana Coal Resources. WALTER A. HASENMUELLER, LOUIS V. MILLER AND JIMMY J. JOHNSON, Indiana Geological Survey, Bloomington, Indiana 47405.——Sulfur occurs in Indiana coal as sulfides (pyritic sulfur), sulfates, and organic compounds, each of which behaves differently in various physical and chemical coal-cleaning processes. We compiled sulfur-forms data (moisture-ash free basis) on 189 complete channel samples of Indiana coal to assess the distribution of sulfur forms. Pyritic sulfur ranges from less than 0.01 to 8.2 percent and averages 2.1 percent, organic sulfur ranges from 0.2 to 7.2 percent and averages 1.6 percent, and sulfate sulfur ranges from less than 0.01 to 3.0 percent and averages 0.4 percent. Average inorganic sulfur (pyritic plus sulfate) is highest (5.0 percent) in unnamed coals in the Staunton Formation and lowest (0.6 percent) in the Lower Block Coal Member (Brazil Formation). Average organic sulfur is highest (4.6 percent) in the Colchester Coal Member (Linton Formation) and lowest (0.6 percent) in the Lower Block Coal Member. Inorganic sulfur correlates moderately well with organic sulfur ($r = 0.4874$). The broad ranges and large variance of sulfur-forms data and the poor correlation between sulfur forms suggest complex geologic controls. A larger data base is required to advance interpretation beyond this general assessment.

Relation of Petrology to SO_2 Absorption of Carbonate Rocks from Southern Indiana. N.R. SHAFFER AND L.J. KRAUSE, Indiana Geological Survey, 611 N. Walnut Grove, Bloomington, Indiana 47405.——Wet scrubbing of SO_2 from stack gas using limestone slurries is the most common technique for complying with EPA restrictions at existing powerplants. Many chemically similar stones, however, show different capacities for absorbing SO_2. We collected, according to lithology, 170 carbonate-rock samples from southern Indiana to determine if petrology related to SO_2 absorption capacity, as measured by an empirical relative-reactivity test.

Physical properties of absorption (0.4 to 11.8), specific gravity, and insoluble

residue (0.3 to 35.4, average 6.8%) plus detailed petrographic point counts showed the following relations to reactivity values: There appear to be poor correlations of gross absorption or specific gravity with reactivity and a negative correlation (−0.43) of insoluble residue with reactivity. Petrographic percentages of dolomite (−0.52), clay (−0.47), quartz (−0.39), and porosity (−0.38) showed significantly negative correlations with reactivities. Calcite showed worse correlations. Petrographic information, though no panacea, can aid in assessing suitability of limestones for SO_2 scrubbing.

Sulfur Forms, Trace Elements and Mineralogy of the Danville Coal Member (VII) in Indiana. Randall E. Taylor and Haydn H. Murray, Department of Geology, Indiana University, Bloomington, Indiana 47405.——The Danville Coal Member (VII) occurs at the top of the Pennsylvanian age Dugger Formation. This coal ranges in thickness from 0.2 to 6.5 feet, averages 12,200 BTU per pound, 12.5% ash, and 1.5% sulfur. Operating mines are found from Vermillion to Warrick Counties. The pyritic sulfur averages 0.67%, the sulfate sulfur 0.02%, and the organic sulfur 0.81%. Elements more concentrated in the Danville coal than in other Illinois basin coals are Ba, Ni, Al, Mg, Na, K, and Ti. Mineral matter in the Danville coal are kaolinite, illite, quartz, and pyrite along with traces of calcite, chlorite, smectite, and gypsum. The relatively low sulfur content of the Danville coal is attributable to a combination of being overlain in many areas by a thick non-marine shale unit and its depositional position on the upper portion of a deltaic platform.

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A Study of Virgilian Sediments in Coles County, Illinois— The Indian Creek Section, Mattoon Formation

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Introduction

The Mattoon Formation (McLeansboro Group) is the youngest unit of Pennsylvanian age in Illinois (Fig. 1). The formation ranges in age from late Missourian

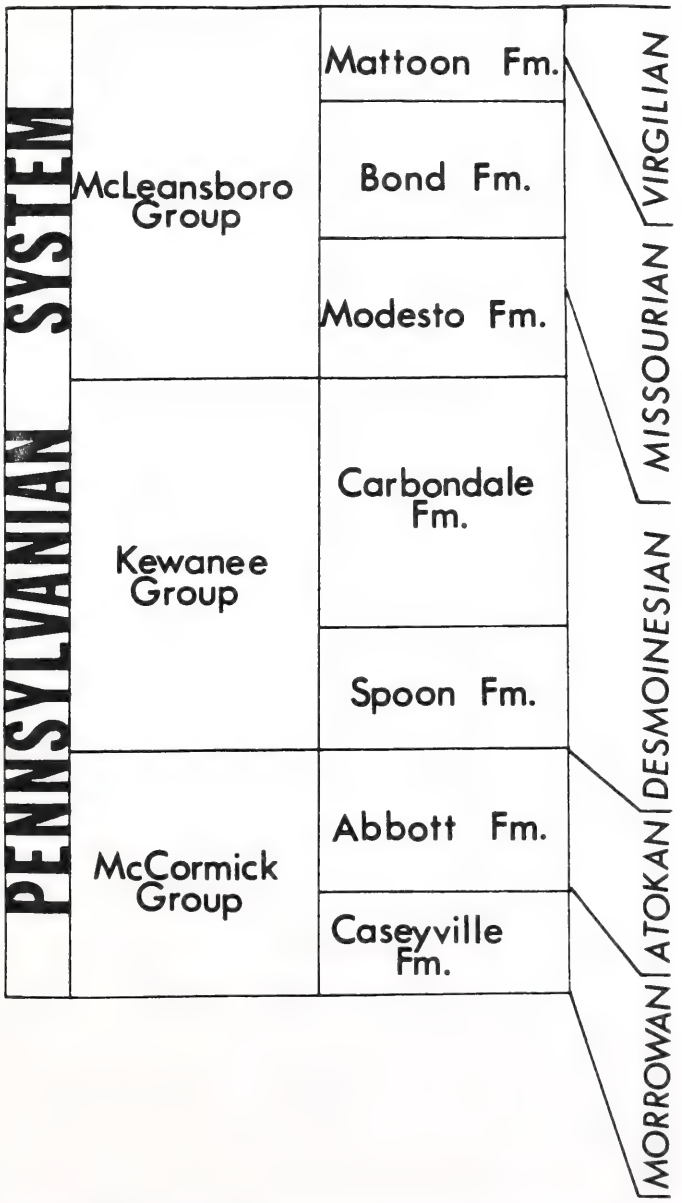

Figure 1. Stratigraphic column of the Pennsylvanian System in Illinois (modified from 3).

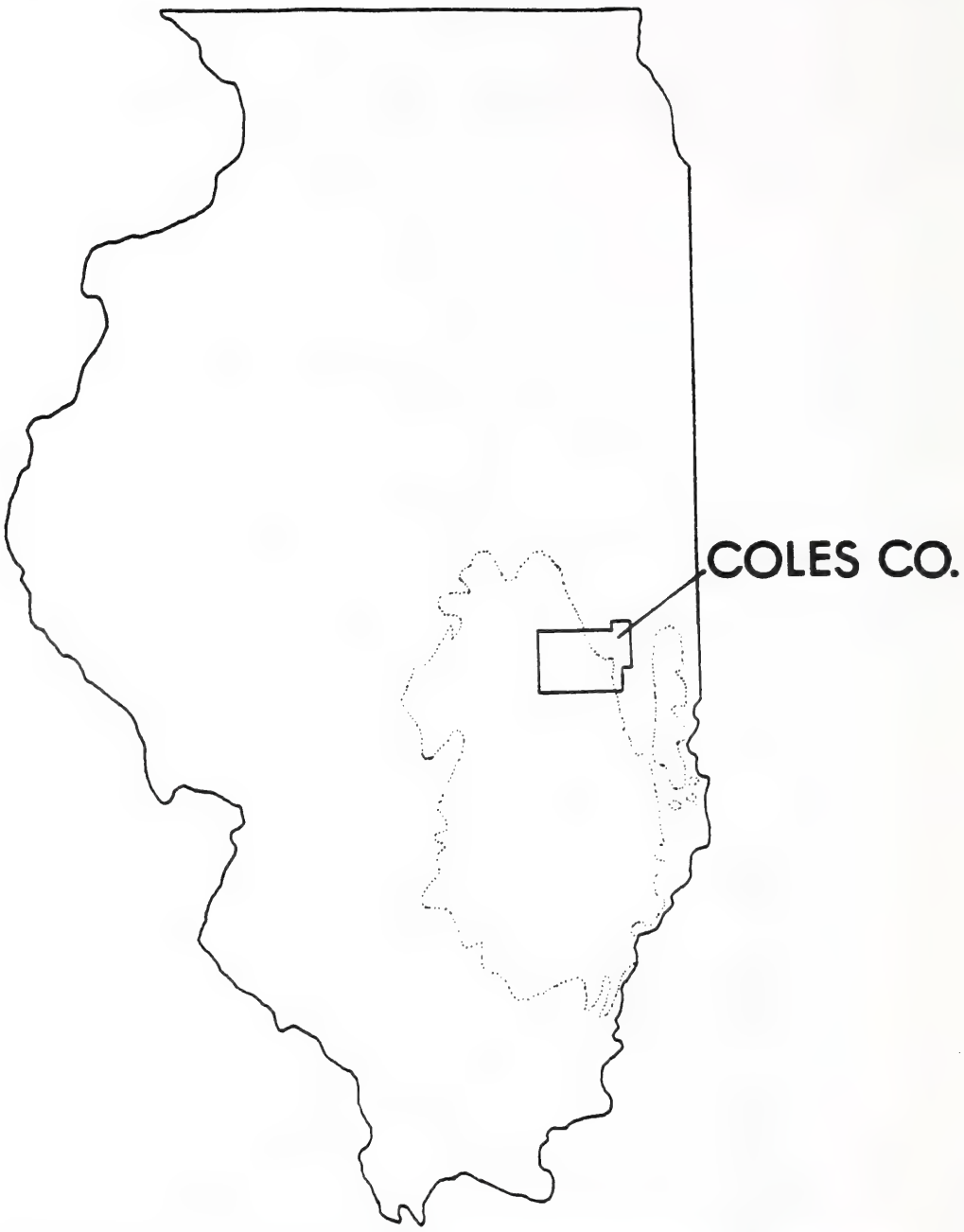

FIGURE 2. Location of Coles County, Illinois. Dotted line shows known extent of Mattoon Formation in southeastern Illinois (based on 17).

through Virgilian in age. Continous surface exposures are limited, owing to thick sequences of glacial drift which obscure the formation. In many areas, post-Pennsylvanian erosion has removed the formation entirely. As a result, major units are difficult to trace for any distance, and depositional and stratigraphic knowledge is limited. Paleontologic work, for the most part, has been limited to limestone members within the formation (3, 11, 16, 17). Though correlation of surface exposures with subsurface data has proven difficult, Wiebel (15) has been able to correlate black shales associated with some limestone members.

The objective of this study is to provide previously unpublished data about the fauna and depositional history of Virginian-age sediments in the Illinois Basin.

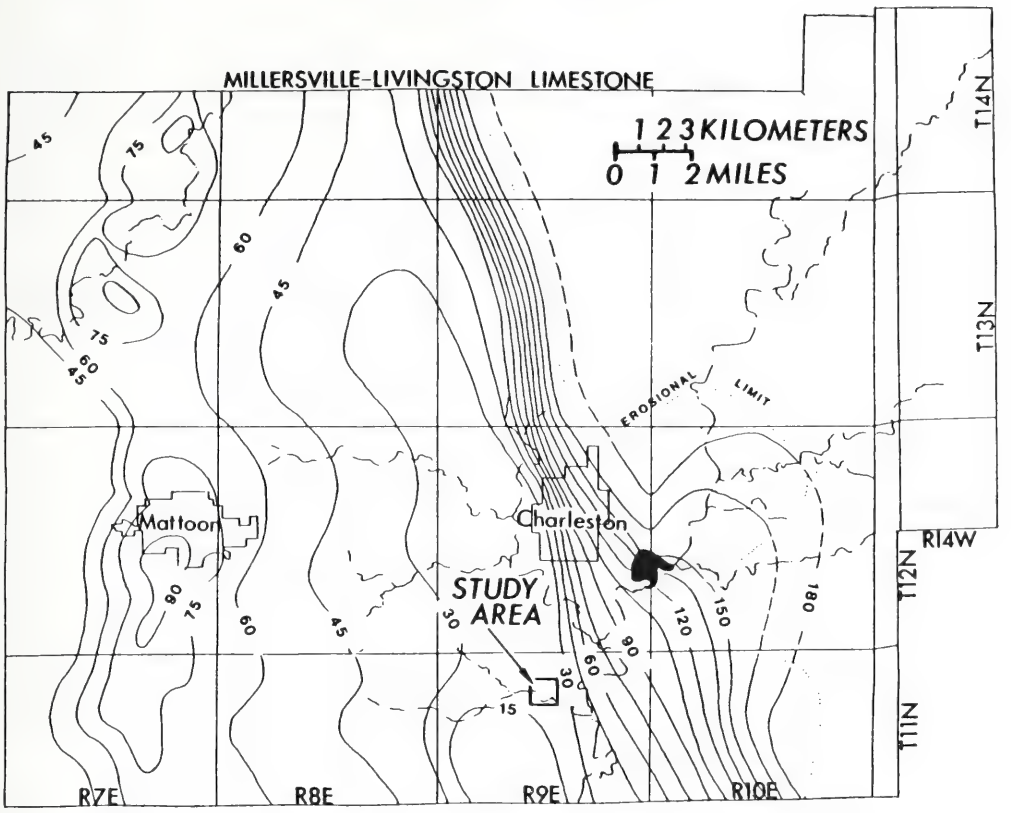

FIGURE 3. Structure on top of the Livingston-Millersville Limestones (top of the Bond Formation, base of the Mattoon Formation), Coles County, Illinois. Based on data at the Oil and Gas Section, Illinois State Geological Survey, Champaign, Illinois. Contour interval is 15 meters (50 feet).

Geologic Setting

The Indian Creek section, Section 10, T11N, R9E, is one of several sections of the Mattoon Formation exposed along the Embarras River and its tributaries in southern Coles County, Illinois (Fig. 2 and 3). These sections are conveniently located on the steep west flank of the LaSalle Anticlinal Belt (Fig. 3), which allows study of the formation at several points ranging from just above the Livingston-Millersville Limestone to the Indian Creek section, over 151 meters above the base of the formation. The Mattoon Formation reaches a maximum thickness of 181 meters in the subsurface of southeastern Illinois (17), hence the Indian Creek section may be the youngest Pennsylvanian sediments yet studied in Illinois. The high position of the section within the formation places it well above the Omega Limestone, the approximate location of the Missourian-Virgilian boundary (16).

Stratigraphy and Paleontology

The Indian Creek section consists of six separate stratigraphic units (Fig. 4). Unit 1 is a light gray, calcareous underclay overlain by a medium gray to brownish black, badly leached coal. Unit 2 is a gradational sequence of dark to medium light gray shales which are in sharp contact with the underlying unit. The shales form a resistant ledge at the bottom of the sequence which displays tight, thin fissility. The fissility decreases upward. Toward the top of Unit 2, a zone of ironstone concretions occurs, above which fissility is once again developed. The shales are calcareous

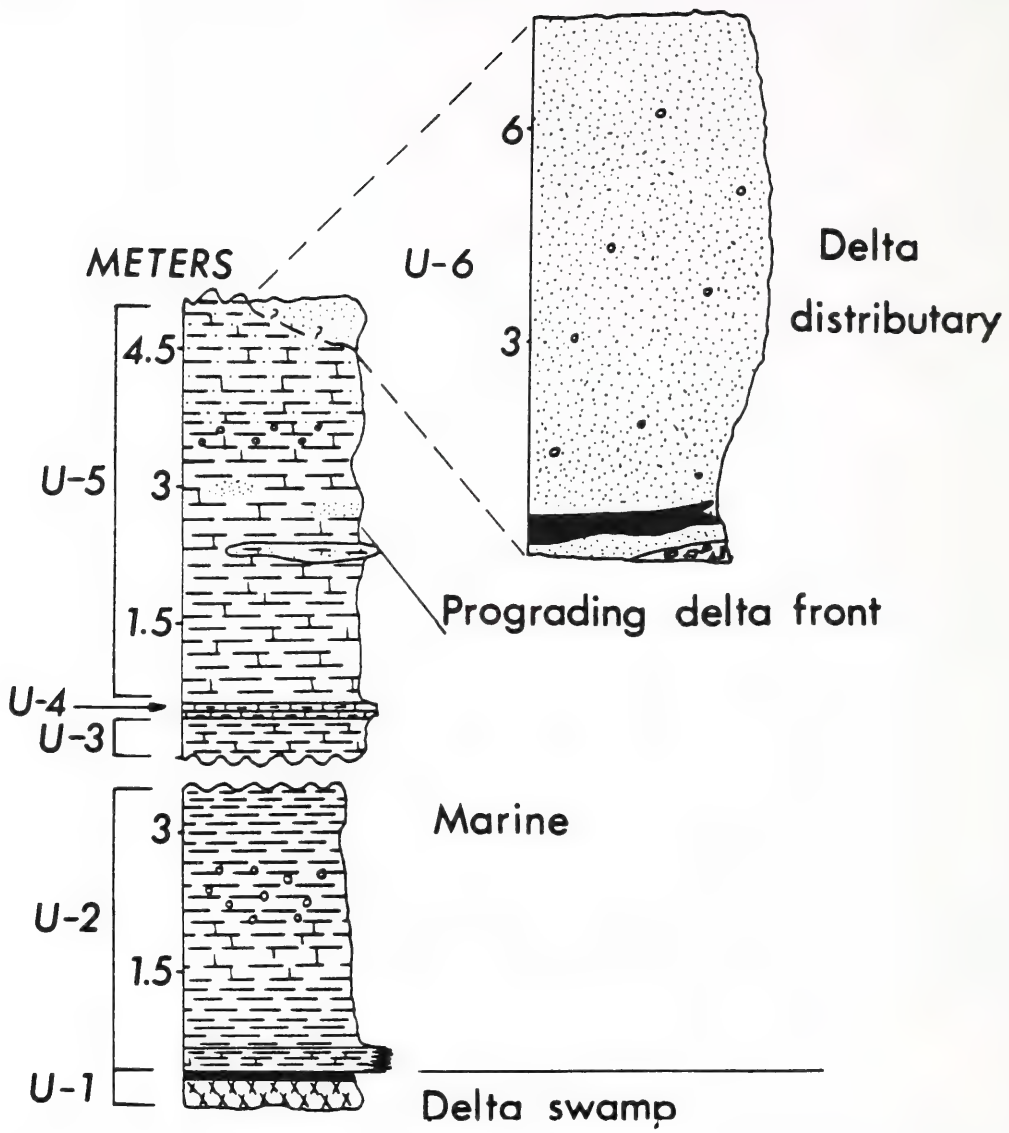

FIGURE 4. Indian Creek section, section 10, T11N, R9E. U-# indicates stratigraphic units described in text.

below the concretion zone, but lack carbonate content at the top of the unit. Units 3, 4, 5 and 6 occur in separate exposures, with any intervening strata missing.

Unit 3 is a dark gray, calcareous, carbonaceous to coaly shale which splits into fissile or platy sheets. Numerous microscopic pyrite nodules can be seen in disaggregated samples. Burrow mottling is evident throughout the shale. A light gray, micritic limestone, Unit 4, overlies Unit 3. The limestone displays vague parallel alignment of calcite crystals in thin section. Calcite veins and silty layers containing muscovite and carbonaceous matter part the limestone. The unit is thin but laterally continuous in exposure. Unit 5 is a gradational sequence of light gray shales which vary from thin bedded to fissile. Muscovite, carbonaceous debris and plant fragments are abundant. A thin, light gray sheet sandstone occurs in the lower part, forming a flat ledge that is laterally discontinuous. An ironstone concretion zone occurs toward the top of the shale sequence. Sandy lenses or patches are developed in the middle and upper parts of Unit 5. Carbonate content is lost as the unit coarsens upward.

Unit 6 is a thick, light gray to grayish-orange sandstone. The sandstone varies

TABLE 1. Paleontology of Indian Creek Section

Unit	Type of organism	No. of specimens-Genus species
2	Brachiopods	productids, productid spines
		unidentifiable
	Bryozoans	Fenestellid fragments
	Cephalopod	unidentifiable
	Conodonts	1-*Hindeodella* sp.
		14-*Streptognathodus* sp.
	Crinoids	arm plates, axialaries, stems, radial plates
	Foraminifera	1-*Pseudoammodiscus* sp.
	Gastropods	4-*Porcellia* sp.
	Holothuroids	*Eocaudina* sp. fragments
		2-*Paleochirodota* sp.
	Microcrinoid	*Kallimorphocrinus* sp. fragments
	Starfish	Ophiuroid fragments
	Trilobites	spines and fragments
	Echinoids	spines and plates
	Spores	
3	Brachiopods	1-*Phricodothyris perplexa*?
		unidentifiable
	Conodonts	1-*Spathonathodus minutus*
		3-*Streptognathodus* sp.
		1-*Streptognathodus elegantulus*
		4-*Adetognathus sp.*
		5-*Hindeodella* sp.
		1-*Ozarkodina* sp.
	Foraminifera	1-*Cyclogyra* sp.
		1-*Endothyra* sp.
		2-*Endothyranella* sp.
	Gastropods	1-*Donaldina* sp.
		2-*Streptacis* sp.
	Holothuroids	1-*Eocaudina* sp.
		1-*Eocaudina gutschicki*
		6-*Eocaudina mccormacki*
		2-*Paleochiridota* sp.
	Ostracodes	6-*Bairdia* sp.
		2-*Cavellina* sp.
		7-*Pseudobythocypris* sp.
		3-*Healdia* sp.
		8-*Amphissites* sp.
	Crinoids	stems and arm plates
	Fish	teeth
4	Bivalves	unidentified
5	Condonts	14-*Streptognathodus* sp.
		1-*Streptoganthodus gracilis*
		2-*Adetognathus* sp.
	Foraminifera	1-*Endothyra* sp.
	Gastropod	1-*Porcellia peoriensis*?
	Ostracodes	1-*Amphissites* sp.
		2-*Cavellina* sp.
		1-*Healdia* sp.
	Brachiopods	productid spines and fragments
	Crinoids	arm plates and stems
	Spores	

from lithic arenite to sublitharenite, with a basal conglomerate, faint parallel laminations, and ironstone concretions oriented randomly irrespective of bedding. A coal lense occurs near the base of the sandstone.

Invertebrate organisms recovered from the Indian Creek section are listed in Table 1. Disaggregation of the shales yielded 24 genera of marine fossils, such as

conodonts, crinoids, brachiopods, foraminifera, microgastropods, holothuroids and ostracods. In addition, spores, fish teeth, a cephalopod, and fragments of crinoids, echinoids and brachiopods were found. Bivalves observed in Unit 4 were unidentifiable.

Environment of Deposition

The abundance of marine fossils (Table 1) proves that many of the rocks exposed at Indian Creek were deposited primarily in marine environments. Deposition seldom occurred far from shore, however, evidenced by the high carbonaceous content of the shales (4).

Several notable differences exist between marine fossils in Unit 2 and marine fossils in Units 3 and 5 (Fig. 4). Unit 2 shales contain the remains of fenestellid bryozoans, *Kallimorphocrinus*, and ophiuroid starfish; similar remains are missing in Units 3 and 5. On the other hand, ostracodes are numerous in some parts of the shale sequence but missing elsewhere. The presence and absence of certain marine fossils reflects changes that took place in the marine environment.

The presence of an underclay and thin coal at the base of the section suggests nonmarine environments (Fig. 4). The underclay is typically calcareous and lacks lamination. Lack of lamination can be due to 1) reorientation of micaceous clay mineral flakes, owing to root penetration, 2) massive flocculation of clay suspended in transport, owing to initial contact with brackish water electrolytes in a lagoon, delta setting or estuary, or 3) flocculation of clay in an aqueous medium which was drained before sufficient loading could cause consolidation or reorientation of clay flakes (13). No such zone is evident in the underclay, but the overlying thin coal is severly leached. Leaching may be due to either exposure by marine regression, or exposure above groundwater level (13). Therefore, subaerial exposure did occur prior to marine transgression.

The overlying shale at the base of Unit 2 was deposited by an influx of fresh or brackish waters. Parallel, fissile stratification implies that deposition took place in very quiet waters devoid of bottom currents. The high carbonaceous content may have created a reducing environment which prevented colonization by marine or brackish water organisms. Since bioturbation was lacking, fissility was preserved (8). Marine fossils are numerous in Unit 2. A great diversity of benthic and planktic forms are present. The presence of fenestellid bryozoans suggests that bottom waters were slowly circulated during deposition. Flume studies have revealed that the fenestelled genus *Polypora* fed from bottom currents that circulated at one to three centimeters per second. Faster velocities could not have occurred without damaging or breaking the feeding curtain. In addition, these currents were unidirectional (10). Ophiuroid starfish and crinoids also may have benefitted from bottom currents. Other bottom dwellers included productids, holothuroids and trilobites. Conodonts and foraminifera occupied the overlying waters. Ostracodes, usually quite common in marine strata, are missing in Unit 2. They do occur in association with conodonts and forminifera in Units 3 and 5; therefore, their absence from Unit 2 is peculiar, and implies that an environmental factor was present at the time of deposition which discouraged them from inhabiting the area. A lack of plant fragments suggests that direct terrigenous input from nearby deltas was weak. Fissility is also missing, owing to bioturbation and burrowing by organisms. Ironstone concretions and a lack of calcite at the top of the lower shale sequence suggests calcareous organisms were absent and deposition took place in poorly circulated, poorly oxygenated waters (8).

Unit 3 contains numerous and diverse microfossils, both benthic and planktic. Pyrite nodules and a large carbonaceous content suggest that deposition took place under strong reducing conditions brought on by decay of organic matter in quiet

waters (4, 8). However, microfossil content and burrow mottling proves that organisms thrived on the ancient substrate, such as holothuroids and microgastropods (Table 1). Fenestellid bryozoan and starfish fossils are missing, indicating the ancient marine waters were quiet and poorly circulated. Overlying marine waters possessed abundant marine life, especially conodonts, foraminifera and ostracodes. The large frequency of conodonts reflects slow rates of sedimentation (5). The presence of the conodont *Adetognathus* (*Cavusgnathus* of Merrill) indicates a regressive, nearshore, shallow marine environment existed (5). The absence of productids also may imply shallow water deposition. For example, Stevens (9) discovered a great increase in brachiopod diversity occurred at depths of 15 or more meters, whereas very few brachiopods lived at shallower depths. Absence of brachiopods also may indicate lack of a muddy substrate (Horowitz, pers. comm.).

The presence of Unit 4, a limestone, indicates a significant but temporary change in the marine environment. Terrigenous imput was either weak or absent, allowing carbonate production to occur. The presence of bivalves, who are filter-feeders, also indicates that marine waters were clear and far removed from terrigenous imput. The limestone consists mostly of lime muds deposited in a calm, well oxygenated, low energy environment. The marine setting may have been lagoonal, since modern-day carbonate muds are rarely deposited in areas of a shelf open to oceanic influence (18). Silty partings present in the limestone may be debris washed into the local area by storms. This marine carbonate setting is short-lived, however, and the reason for its termination is apparent in Unit 5.

Fossils reflect less diversity in Unit 5 (Table 1). Productids are present again, but bottom dwelling holothuroids are missing. *Adetognathus* indicates nearshore conditions were present. Unit 5 exhibits an influx of silt and sand, an ironstone concretion zone, a coarsening upward sequence and a decrease in calcite content, all indicative of a prograding delta (2, 14).

The association of the sandstone facies, Unit 6, with a prograding delta sequence implies a deltaic origin. Poor to moderate textural and mineralogical maturity, poor sorting, coarse grain size, high carbonaceous content, enormous thickness, and a conglomerate at the base are characteristics of an elongate sandstone channel of fluvial-distributary origin (6, 7). Lenses of coal, such as the coal at the base, are common in distributary channels (14). Except for faint parallel laminations, the sandstone is massive. Other sedimentary structures are absent or poorly preserved. Massive sandstones deposited in a fluvial-distributary channel may occur at the top of a prograding delta sequence (2).

Conclusions

The Indian Creek section provides a view of the fauna and depositional history of little-known Virgilian-age sediments in the Illinois Basin. Rocks exposed along Indian Creek were deposited by a marine transgression, followed by a marine regression and delta progradation. The basal units were deposited by a marine transgression which flooded a coal swamp. Transgression probably resulted from delta shift or abandonment and subsequent advance of marine waters. Anoxic water conditions alternated with open, shallow water marine conditions. The point of maximum transgression is probably marked by the deposition of carbonate muds, far from terrestrial imput. The shales which followed were deposited in a nearshore, shallow water, regressive marine environment. Marine regression was a direct response to delta progradation into the area. A delta platform was established, across which distributary channels flowed. The marine-deltaic nature of the Indian Creek section is similar to previous assessments of the Virgilian Epoch in the Illinois Basin (1, 12).

Acknowledgments

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The Effects of the Formation of the Wadsworth Sinkhole on the Drainage of Wadsworth and Landreth Hollows, South Central Indiana

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Abstract

The karst area of south-central Indiana contains many allogenic valleys, including Landreth and Wadsworth Hollows, which are located about 6 km north of Orangeville, Indiana. The development of these valleys is in response to solution of the Ste. Genevieve and Paoli Limestones which lie below the much less soluble or nonsoluble rocks of the Chester Series and Mansfield Formation. Sinkholes have formed in the dissolving limestone, diverting surface drainage underground.

The effects of the formation of the Wadsworth Sinkhole on surface drainage in the area were assessed through the study of stratigraphic sections exposed along the walls of Wadsworth Hollow. Imbrication of clasts, along with the topography of the bedrock surface, suggest that a former stream channel flowed to the northeast through the area. The slope of the bedrock surface and the lack of alluvial sediments in certain areas suggest that the collapse of the sinkhole may have initiated several episodes of rapid erosion and sediment influx.

Introduction

Allogenic valleys (sometimes called "hollows" on topographic maps) are common along the boundary between the Crawford Upland and Mitchell Plain in south-central Indiana where nonsoluble clastic rocks of the Mansfield Formation and Chester Series overly the middle Mississippian Ste. Genevieve Limestone and Paoli Limestone. The clastic rocks form the uplands, whose surface drainage is diverted into sinkholes along the floors of the allogenic valleys where the limestone is at or near the surface. Streams no longer flow along these valleys except during periods of excessive rainfall and runoff from the surrounding uplands.

During heavy runoff events, the flow of two such allogenic valleys, Landreth and Wadsworth Hollows, is presently diverted into the Wadsworth Sinkhole, a large collapsed sinkhole (Figures 1 and 2). However, inspection of the topographic map of the Wadsworth drainage basin (Figure 1) suggests that the drainage from these valleys formerly merged and flowed north. The water from the Wadsworth Sinkhole rises at Sulfur Creek, approximately five miles southeast of the study area (1).

The purpose of this study was to determine how the formation of the Wadsworth Sinkhole altered the surface drainage in the study area.

Methods

Alluvial deposits along Landreth and Wadsworth Hollows were examined for evidence that might indicate the direction of current flow before collapse of the sinkhole altered the surface drainage. Tabular clasts along the present stream channel show a pronounced imbrication that indicates the direction of present current flow (Figures 2 and 3, Location 4). Thus, it seemed reasonable that former directions of flow might be revealed by clast imbrication within older alluvial deposits along the channels.

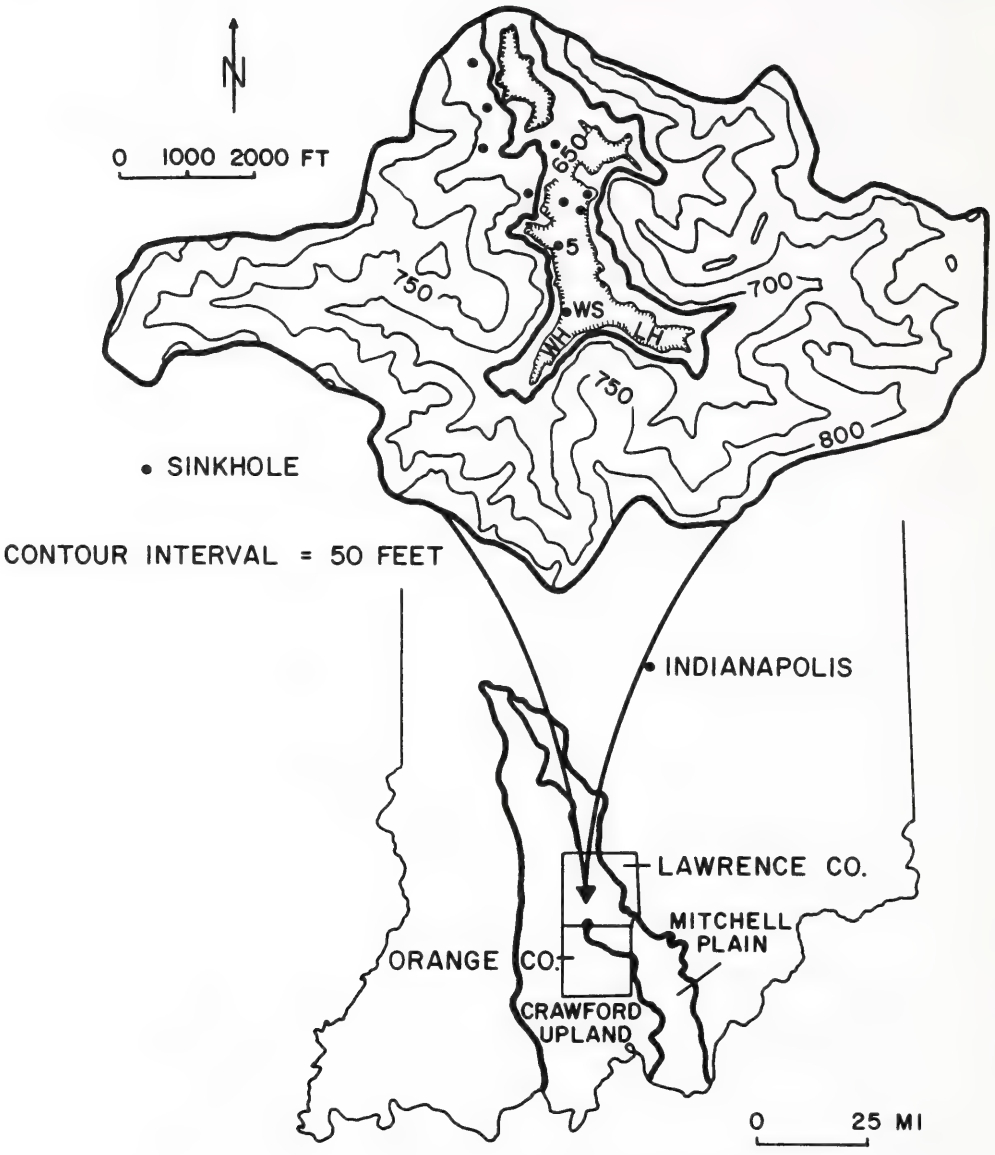

FIGURE 1. Location map of study area and topographic map of the Wadsworth Hollow drainage basin, modified from U.S. Geological Survey 7.5-minute Georgia, Indiana, Quadrangle. The heavy solid line on the drainage-basin map is the contact between the Ste. Genevieve and Paoli Limestones in the valleys and the overlying clastics of the Chester Series and the Mansfield Formation. LH-Landreth Hollow, WH-Wadsworth Hollow, WS—Wadsworth Sinkhole, 5-location of stratigraphic section 5 (See text).

Stratigraphic sections were investigated at selected locations along channel walls, including digging below the level of the modern stream channel (Figure 2, Locations 1, 2, and 3; Figure 1, Location 5). The attitudes of tabular clasts from the deposits at these locations were measured and converted to azimuths in the directions of current flow (Figure 3). Such data were collected for thirty clasts from each sampled unit.

The sample locations were generally restricted to areas where good exposures of alluvial sediments were present along the channel walls. Many areas along the channel have experienced slope failure, expecially near the sinkhole, rendering impossible the collection of clast-orientation data.

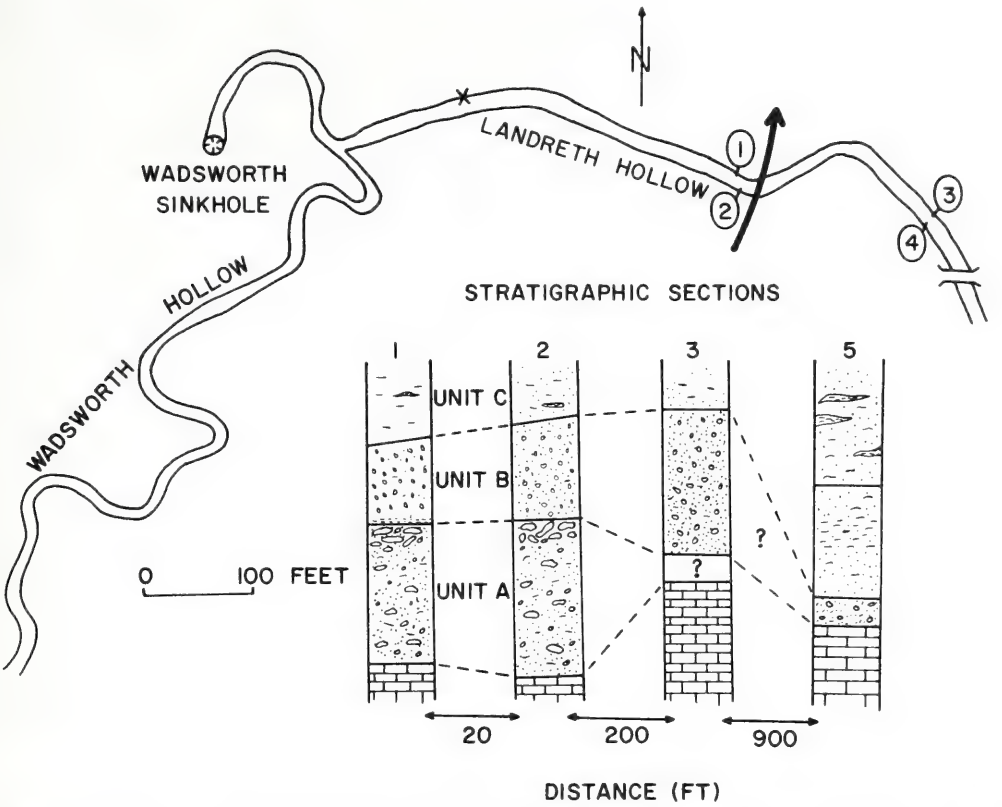

FIGURE 2. Drainage map of the study area showing locations of sampled stratigraphic sections 1 through 3 and stream-bed location 4 (circled), location of the stratigraphic section near Wadsworth Sinkhole in which no clasts were found (x), and inferred paleodrainage route (heavy arrow). Correlation diagram of stratigraphic sections 1 through 3 and 5 (for the location of the latter, see Figure 1).

Data Analysis

Because of the restrictions noted above, the closest stratigraphic section to the Wadsworth Sinkhole from which clast-orientation data could be obtained is Location 1 (Figure 2). In a stratigraphic section closer to the sinkhole (marked with an x in Figure 2) no clasts were found above bedrock. At Location 1 two clast-bearing stratigraphic units were found. Clasts from the upper Unit B reveal a westerly direction of stream flow (Figure 3), the direction of flow of the present ephemeral stream. However, clasts from the lower Unit A reflect a northeasterly direction of current flow (Fiture 3), although the preferred orientation of clasts in this unit is not as strong as in Unit B. Nevertheless, the data could reflect deposition by a former stream channel with a direction of flow different from the present stream.

Both clast-bearing stratigraphic units are also present at Location 2 slightly upstream along the opposite bank (Figure 2). Clasts from Unit B reflect a westerly to northwesterly direction of current flow, but for the underlying Unit A a northeasterly direction is indicated (Figure 3). The preferred orientation of clasts in Unit A at Location 2 is much stronger than at Location 1.

At Location 2 the stratigraphic section was excavated to a depth of 1.3 m below the modern stream channel without reaching bedrock. The increased depth to bedrock at this location may support the contention that a former stream channel is present here. We believe that the northeastward-flowing paleochannel was located in the vicinity of Locations 1 and 2 (Figure 2).

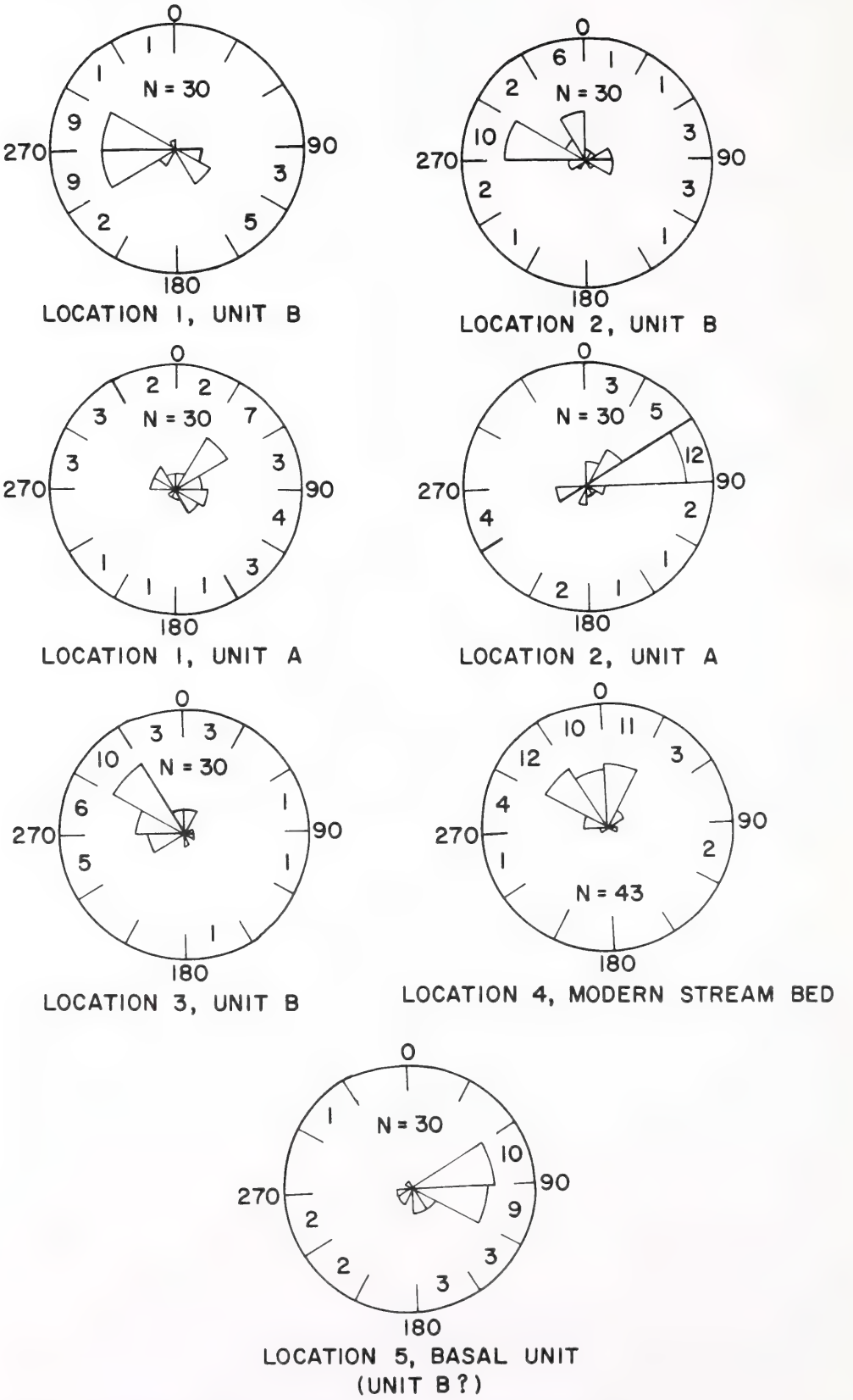

FIGURE 3. Clast-orientation data for stratigraphic sections 1 through 3 and 5 and the stream bed at Location 4. N-number of clasts.

At Location 3 slightly downstream from the bridge over Landreth Hollow (Figure 2) only Unit B was encountered, although Unit A may occur below the depth of the excavation. A northwesterly direction of current flow is indicated (Figure 3, Location 3), which compares favorably with clast-orientation data for the present stream channel (Figure 3, Location 4). This area is located far enough into Landreth Hollow that the stream is unlikely to have experienced a drastic change in flow direction.

There are several sinkholes along the valley north of Wadsworth Sinkhole (Figure 1), but only one contains alluvial sediments (Figures 2 and 3, Location 5). Imbrication of clasts indicates an easterly direction of flow, which is obviously contrary to that predicted by the orientation of the valley. However, the flow as indicated by these clasts was probably from a tributary to the west (Figure 1).

We acknowledge that some of the clast-orientation data could be explained in terms of meandering paleochannels. The present channel at Location 4, whose overall flow direction is N32°W, bifurcates into several subchannels that meander somewhat, which is reflected in the scatter of the clast-orientation data (Figure 3). However, we do not believe that channel meandering is an adequate explanation for the relatively strong northeasterly flow pattern as reflected in the data collected from Unit A, especially at Location 2.

Cross sections along Landreth Hollow (Figure 4) and Wadsworth Hollow (Figure 5) show that the contact between bedrock and the alluvial sediments slopes toward Wadsworth Sinkhole. Along Landreth Hollow, the lower contact of the silty uppermost alluvial sediments (Unit C) with underlying Units B and A, if projected toward the sinkhole, terminates against a large outcrop area of limestone bedrock along the stream channel (Figure 4). This outcrop probably marks the rim of the sinkhole.

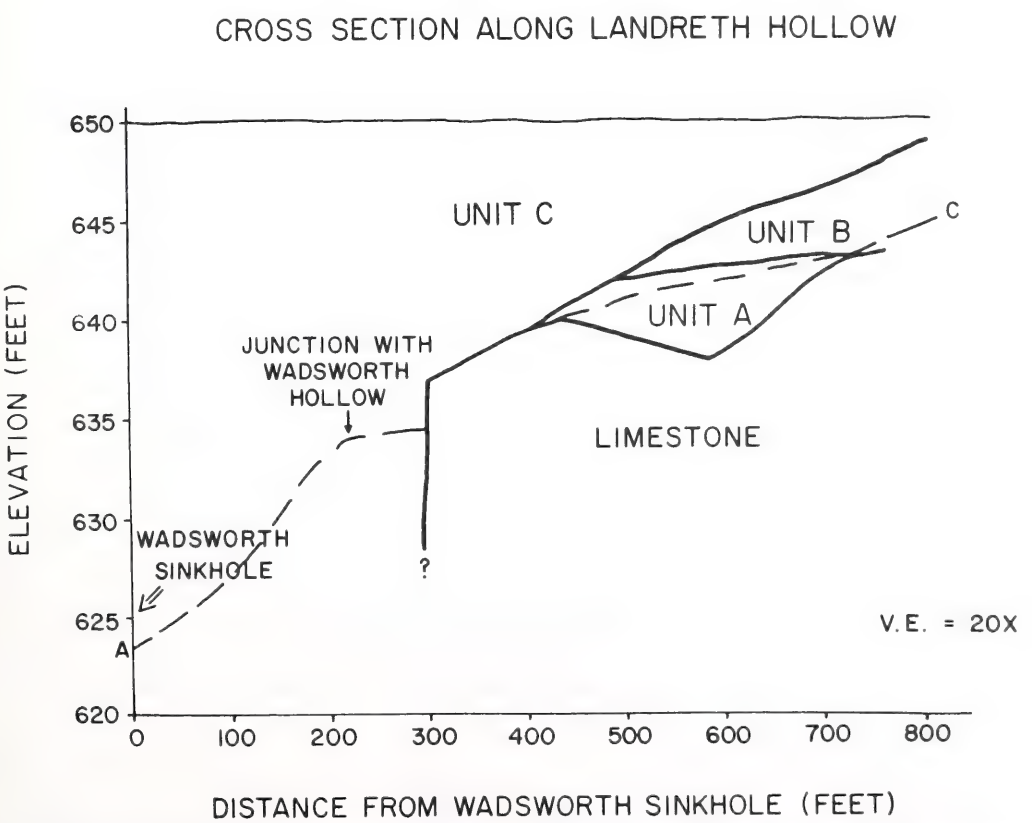

FIGURE 4. Cross section along Landreth Hollow. Dashed line A-C is the position of the stream bed where it does not follow the surface of the limestone outcrop.

When the sinkhole formed by collapse, sediments overlying the bedrock were surely eroded into the sinkhole until this limestone rim was exposed. The rim then probably protected the remainder of Units B and A upstream from further significant erosion. This scenario may explain why no clast-bearing alluvial sediments were encountered in the stratigraphic section marked x (Figure 2) or in the valley north of Wadsworth Sinkhole (Figure 1).

Similar relationships hold for Wadsworth Hollow (Figure 5), but the clast-bearing alluvial sediments are located farther from Wadsworth Sinkhole.

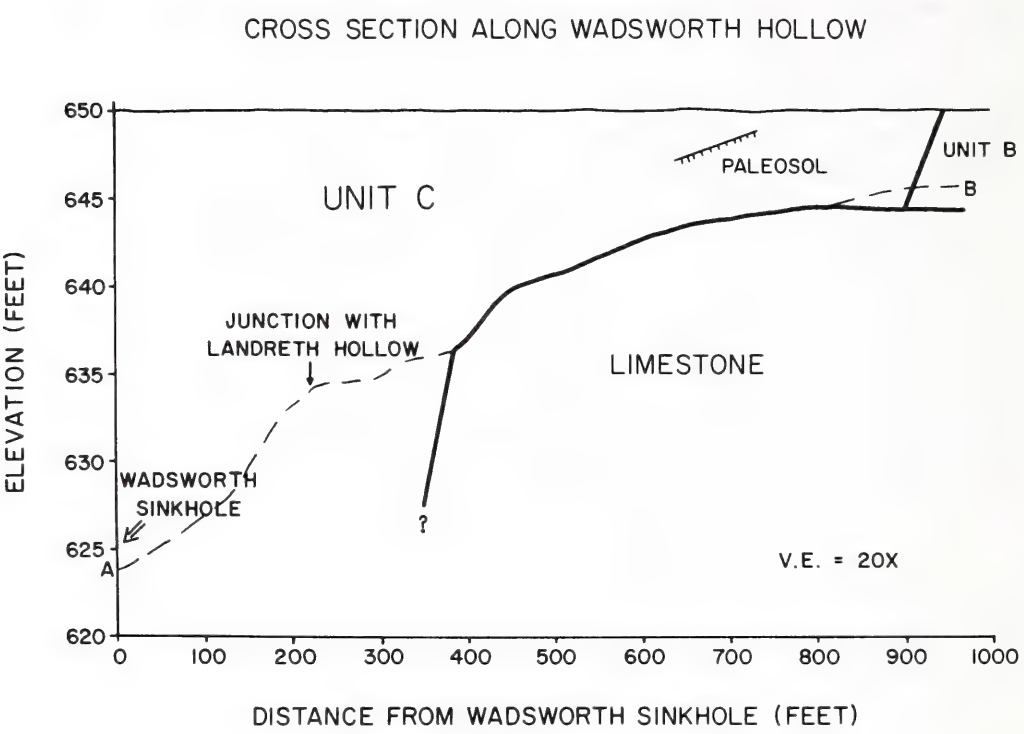

FIGURE 5. Cross section along Wadsworth Hollow. Dashed line A-B is the position of the stream bed where it does not follow the surface of the limestone outcrop.

History of Sinkhole Collapse and Sedimentation

It is likely that the Wadsworth Sinkhole experienced more than one episode of collapse. The first collapse probably rerouted the streamflow from northeastward out of the drainage basin to westward into the sinkhole. The stream exiting Landreth Hollow was diverted toward the sinkhole, and the stream in Wadsworth Hollow was shortened. When the collapse occurred, a strong current stripped much of alluvial Unit A near the sinkhole and transported large quantities of sediment into the sinkhole. When bedrock was exposed at the rim of the sinkhole erosion of Unit A was retarded farther upstream. A zone of very large clasts at the top of Unit A may reflect an erosion surface produced at this time when clasts too large to transport were left as a lag deposit.

Unit B was then deposited, with its clasts at Locations 1 and 2 indicating the new flow direction for the lower part of Landreth Hollow. Overbank deposition occurred frequently in the area adjacent to the channel. Eventually the land surface stabilized, and a soil developed at the top of the overbank deposits (Figure 5).

A second collapse (or possibly reopening of the clogged sinkhole) caused the

soil and alluvial deposits near the sinkhole to be washed into the sinkhole. This interpretation would account for the absence of Unit B near the sinkhole and the sloping lower contact of Unit C toward the sinkhole. Overbank deposition again occurred, eventually burying the soil formed during earlier land-surface stability. A new period of stability ensued, and the present ground soil began to form.

Conclusions

The history of geologic events suggested here is undoubtedly not the only interpretation that can be made from the data presently available. Furthermore, this history is also undoubtedly incomplete, because, in lieu of equipment to cut major trenches through the alluvial sediments of the area, our efforts at data collection are certainly quite limited. Nevertheless, the available stratigraphic evidence suggests that collapse of the Wadsworth Sinkhole did result in reversal of part of the surface drainage of the drainage basin and that sinkhole collapse accompanied by sediment transport into the sinkhole probably occurred on more than one occasion.

The study area experienced alternate periods of sinkhole collapse (or unplugging) followed by overbank deposition, stabilization of the land surface, and soil formation. Regarding the future, it is interesting to note that the present stream flowing through Wadsworth Hollow loses much of its flow into small swallow holes before reaching the Wadsworth Sinkhole. One of these swallow holes may someday collapse, further altering the surface drainage, as has happened in the past.

Acknowledgments

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Paleontology and Paleoecology of Two Pennsylvanian Fossil Sites Near New Harmony, Indiana

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Introduction

The village of New Harmony was the focus of most of the early geological studies in the central United States. The town was the home of David Dale Owen as well as his associates Gerard Troost, William MacClure, F. B. Meek, E. T. Cox and B. F. Shumard, many of whom went on to become state geologists of surrounding midwestern states (12). One consequence of this was that the Pennsylvanian age rocks in the New Harmony area became fairly well known. Studies by McChesney (14,15), Norwood and Pratten (21,22), Meek and Worthen (18,19,20), and Hall (5), which included at least 26 new species from the Pennsylvanian rocks around New Harmony (Appendix 1), were among the earliest publications to deal with the paleontology of North American Paleozoic age rocks. These reports were principally taxonomic descriptions of marine invertebrate fossils.

Since the middle of the 19th century there have been no systematic studies of the old collecting sites in the New Harmony area. The purpose of work leading to this report was to locate original localities, compile a list of species whose type specimens came from these localities, and make modern paleoecological interpretations of the fossils found in the strata at the sites.

Localities

Four New Harmony-area fossil sites were identified from the publications cited above. Three localities, known as "the mouth of Rush Creek", "Big Creek", and the "Wabash Cutoff", are in Posey County, south of New Harmony. The fourth locality is at Grayville, in White County, Illinois (Fig. 1). Only two of the four sites were found, as explained below.

The present mouth of Rush Creek is situated in Harmonie State Park, south of New Harmony (SW 1/4, NW 1/4, Sec. 23, T.5S., R.14W., Solitude 7 1/2 min. quad.). The lower part of Rush Creek is on the Wabash River flood plain and is extensively alluviated. There is some evidence that the mouth of Rush Creek may have shifted through time. Mink Island, just off the mouth of the creek, is not shown on old maps. Thus, the old mouth of Rush Creek may have originally been on the west side of what is now an island. No outcrops of Pennsylvanian rocks were found anywhere in the vicinity of this old locality. Indeed, the outcrop was apparently already slumped and largely covered by the late 1800's (3).

Some uncertainty exists about the exact locality of the Big Creek site. Most references simply call the site "Big Creek", a name that makes any exposure along the creek a candidate for the original locality. According to Collett (3), a fossil site lay "along the road between New Harmony and Mt. Vernon". Today the main road is Indiana State Road 69, but the area where this road crosses Big Creek is overgrown and covered with alluvium. A nearby limestone quarry that was apparently initiated by the Rappites, the early 19th century settlers of New Harmony, as a source of

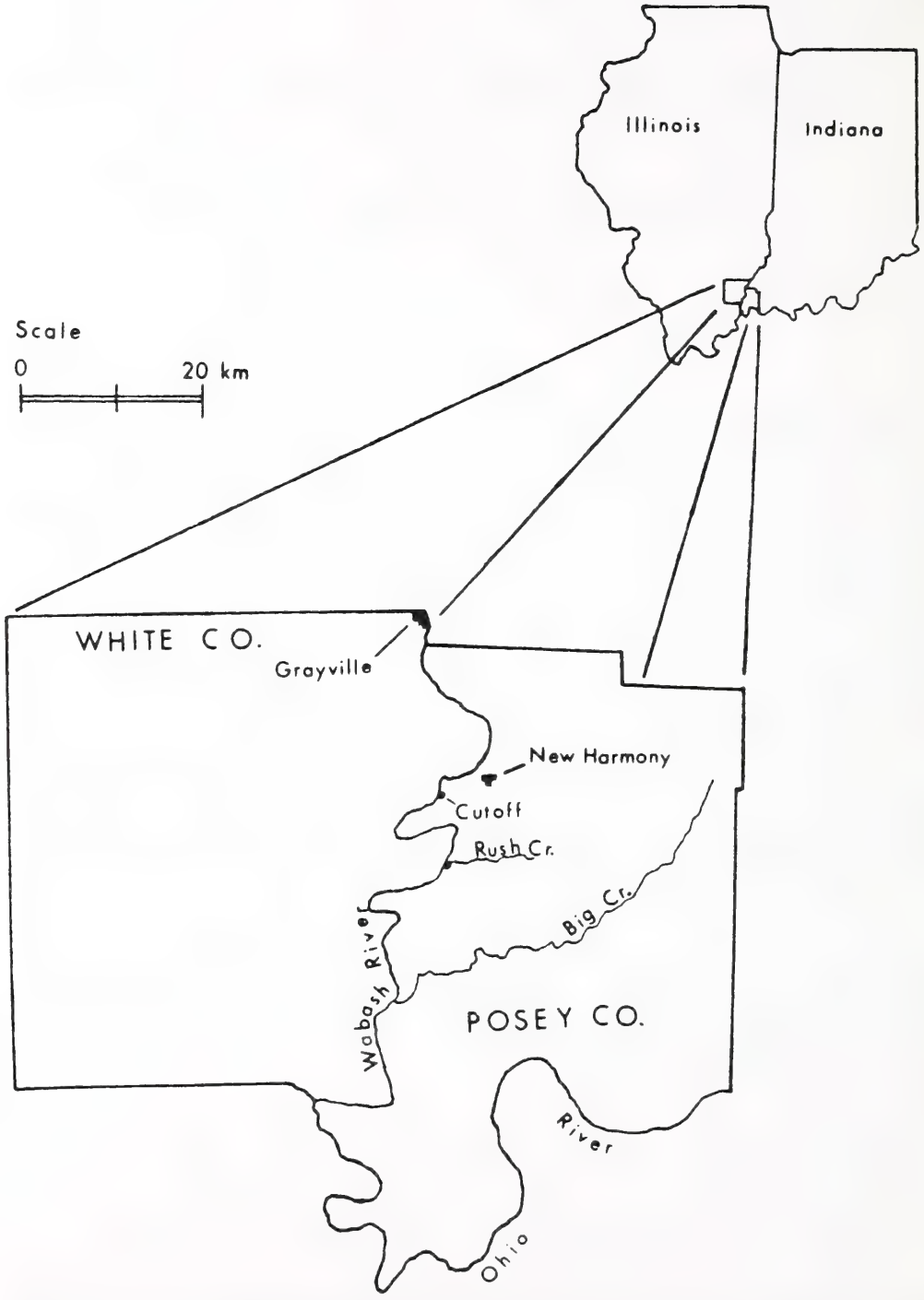

FIGURE 1. Map showing localities of the Grayville and Wabash Cutoff sites as well as the present position of Big Creek and Rush creek.

lime for plaster is now completely filled with sediment and no *in situ* rocks are exposed. This site is a likely, but unproven, candidate for the Big Creek site of early paleontologists. The quarry is situated in the SE 1/4, SE 1/4 Sec. 6, T.6S., R.13W., Solitude Quadrangle (23; locality 771). The old quarry is on the west side of the southeast flowing Solitude Run, and a side road off of SR 69 to a Conservation Club lake is just south of it.

The Wabash cutoff locality is situated on the Wabash River approximately one mile southwest of New Harmony (SE 1/4, NW 1/4, Sec. 11, T.5S., R.14W, Solitude Quadrangle). The thin bed of marine limestone and shale from which collections were obtained is near low water level of the Wabash River and is covered during times of flood. The bed is best studied during Fall months when the river level is low.

The Grayville, Illinois locality is situated along an incompletely cut off meander loop of the Wabash River at Grayville. The site is below the City Park on the southeast edge of Grayville, and along a low bluff below the Illinois Central Railroad tracks (NE 1/4, SW 1/4 Sec. 21, T.3S., R.14W., New Harmony, Ind.-Ill. Quadrangle). The site can be reached by crossing the railroad tracks in the park, driving as far as possible to the north, and then walking down to the river along the foot path. The exposure is completely covered during Spring floods. The meander loop was cut off in the Spring of 1985, but water still flows through the old channel. Presumably the loop will be completely cut off from the main river channel in the future and it is probable that the site will become overgrown in the absence of scouring by high water.

Geologic Setting

Rocks of the New Harmony area were deposited on the eastern margin of the Illinois Basin during Late Pennsylvanian time. Alternation of marine and nonmarine rocks resulted from periods of construction and destruction of a large deltaic complex that prograded across the area from northeast to southwest. Fluctuation between marine and nonmarine conditions resulted in deposition of sedimentary packages termed cyclothems. Correlation of the two statigraphic sequences is made difficult by the fact that they are very similar to many other cyclothems in the Pennsylvanian System as well as the fact that strata are locally disrupted by faults associated with the Wabash Valley Fault System.

The limestone at Grayville has been called the Grayville Limestone (4). This name is not formally accepted by either the Illinois Geological Survey (8) or the Indiana Geological Survey (26), but until better stratigraphic resolution is obtained the name will be used here in a strictly local but unambiguous sense for the marine limestone and shale that crop out at Grayville (Fig. 2). This avoids potentially erroneous cor-

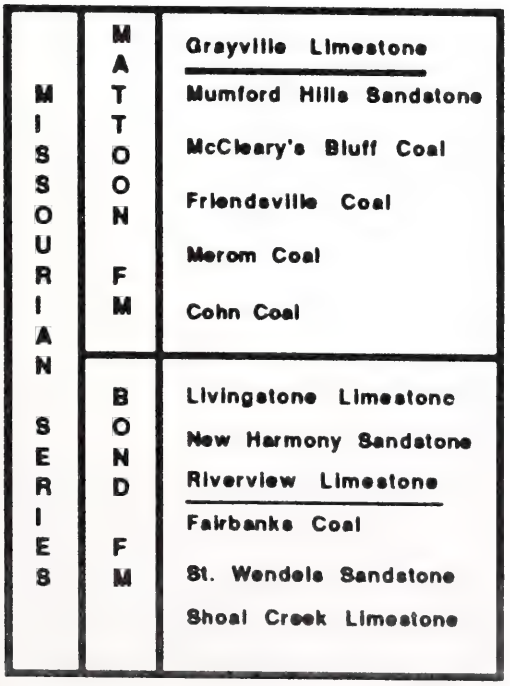

FIGURE 2. Approximate stratigraphic position of the Riverview Limestone and Grayville Limestone.

relation with rocks in other areas. The Grayville interval is usually considered to be in the Mattoon Formation (Missourian; 30).

At Grayville, the limestone is underlain by coal and black shale (Fig. 3). The limestone consists of two distinct beds that are separated, underlain, and overlain

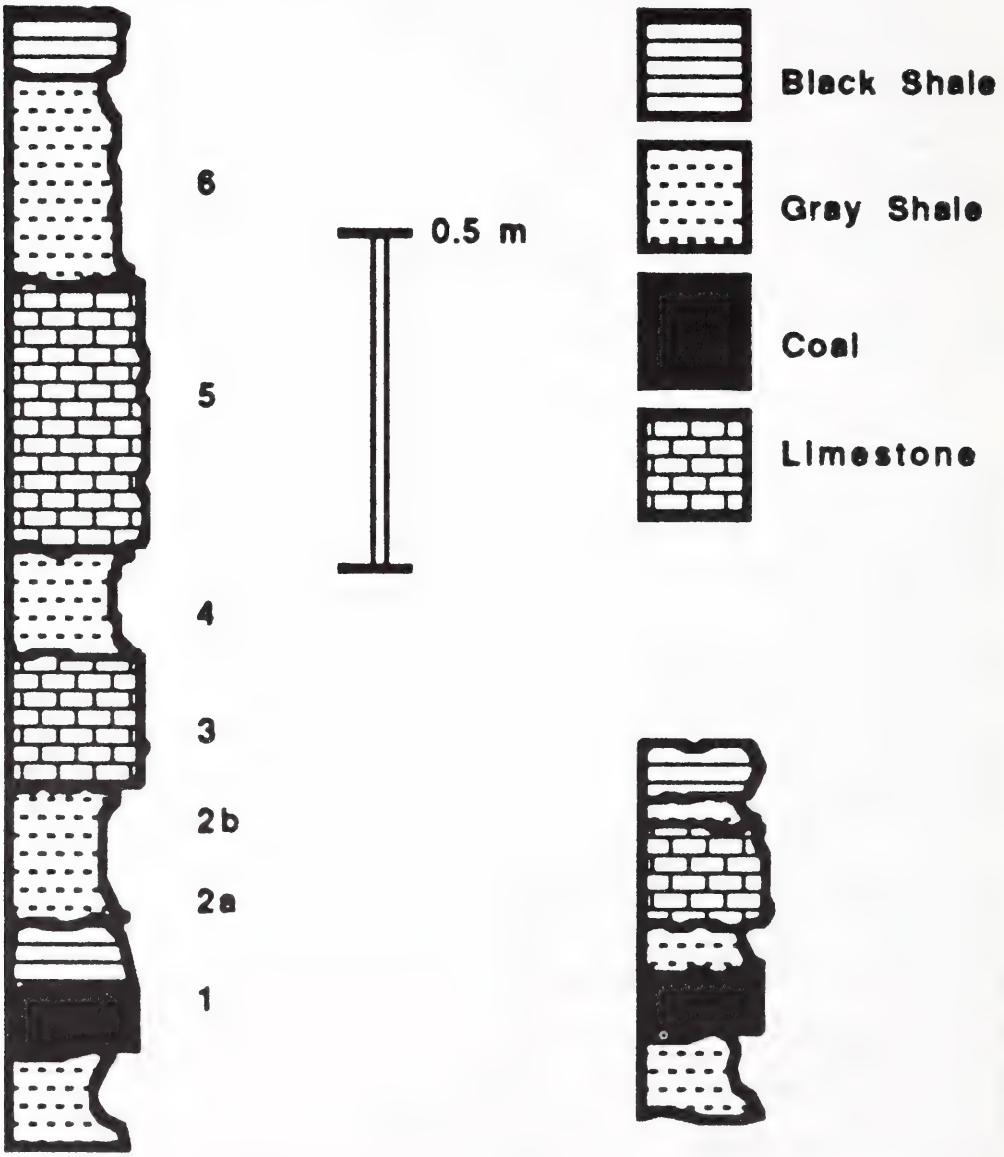

FIGURE 3. Graphic stratigraphic sections of the rocks that crop out at Grayville (left) and the Wabash Cutoff (right).

by calcareous shale. Six lithologic units have been differentiated within the section. Unit 2 has been divided on the basis of fossil content. This sequence of rocks is developed in at least two lense-shaped bodies of rock along the outcrop. All beds thin to near zero thickness from the center of a lense in a distance of 10 to 20 meters.

The limestone at the Wabash cutoff has been identified as the Riverview Limestone Member of the Bond Formation (Fig. 2; 30). Culbertson (4) considered this limestone to be equivalent to the Grayville Limestone on the basis of fossil content and lithology. Differences in fossil content and a statigraphic sequence, however, indicate that the two units are not correlative. A thick sequence of nonmarine sandstone, siltstone

and conglomerate, termed the New Harmony Sandstone, overlies the limestone. A break, based on fossil content, was recognized within the limestone by Wier (30).

Field and Laboratory Methods

Faunal content of shales was determined by examining sieved portions of samples that had been disaggregated by soaking in paint thinner and boiling in water. Limestone and black shale samples could not be disaggregated, thus surface collections from weathered blocks had to suffice. Fossils were identified to generic level in all cases and to the species level when possible. Because shales could be bulk sampled and broken down in the laboratory, there is little chance that important fossil groups were missed, but because the limestone could not be bulk sampled there is a higher probability that some groups, especially microfossils, were missed. As a result, quantitative measurements are limited to classification of taxa as rare or common. Numerical counts, while more desirable, are not used.

Fossil Associations

Mixing of skeletal material between stratigraphically adjacent units is considered to have been minimal. Nevertheless, the term "community" is not used here because of the probability that collecting was incomplete. Instead, faunal groups are called "associations". Seven faunal associations were identified from the rocks of the two sites and are discussed below. The data collected from each unit is shown in Figures 4 and 5.

Dunbarella Association.—A well-defined association that contains the bivalve *Dunbarella whitei*, the brachiopod *Lingula* sp., the worm tube *Spirorbis*, and the ostracode *Geisina arcuata* is present in unit 1 at Grayville. A rare component of the association is the bivalve ?*Anthraconaia*. Fish parts and coprolites are also rare.

The *Dunbarella* association compares favorably with Johnson's (10) group IV assemblage and Hickey and Younker's (7) black shale phase. Components of the association are common in other Pennsylvanian rocks of nonmarine to marginal marine environments. *Dunbarella* is a common form in Pennsylvanian marginal marine facies (17) and may have invaded coastal lakes (16). This bivalve has also been used as an indicator of marine environments in British Upper Carboniferous rocks (2). *Geisina* inhabited fresh water (11) to marine water (2) and was probably euryhaline (24). *Spirorbis* is problematic because it occurs in both marine and nonmarine rocks in the late Paleozoic but is exclusively marine before and after this time interval. *Lingula* is commonly thought of as a marginal marine genus, but is found in marine environments as well.

Septimyalina Association.—A 3-4 cm thick, calcareous bed that overlies the black shale at Grayville is composed almost exclusively of shells of the bivalve *Septimyalina*. Most valves are whole, and many are articulated. *Geisina* and *Spirorbis* are rare. This bed of *Septimyalina* is similar to the *Orthomyalina* bank community of Rollins *et al.* (25), although the fossils reported here form more of a shell pavement and other species are less common. Both of these assemblages resemble modern oyster banks that occur in nearshore areas off of the east coast of North America.

Crurithyris Association.—Two species of the brachiopod *Crurithyris* (*C. planoconvexa* and *C. explanata*) dominate this community. Fenestrate bryozoan fragments are rare to common and *Derbya* is rare. The *Crurithyris* association occurs in the upper part of unit 2 at Grayville.

The mode of occurrence of *Crurityris* conforms to criteria used to recognize opportunistic species (13) and the genus has been found to be opportunistic in Pennsylvanian rocks elsewhere (7). The occurrence of an opportunistic form and the near exclusion of others is generally judged to indicated stressed environmental conditions.

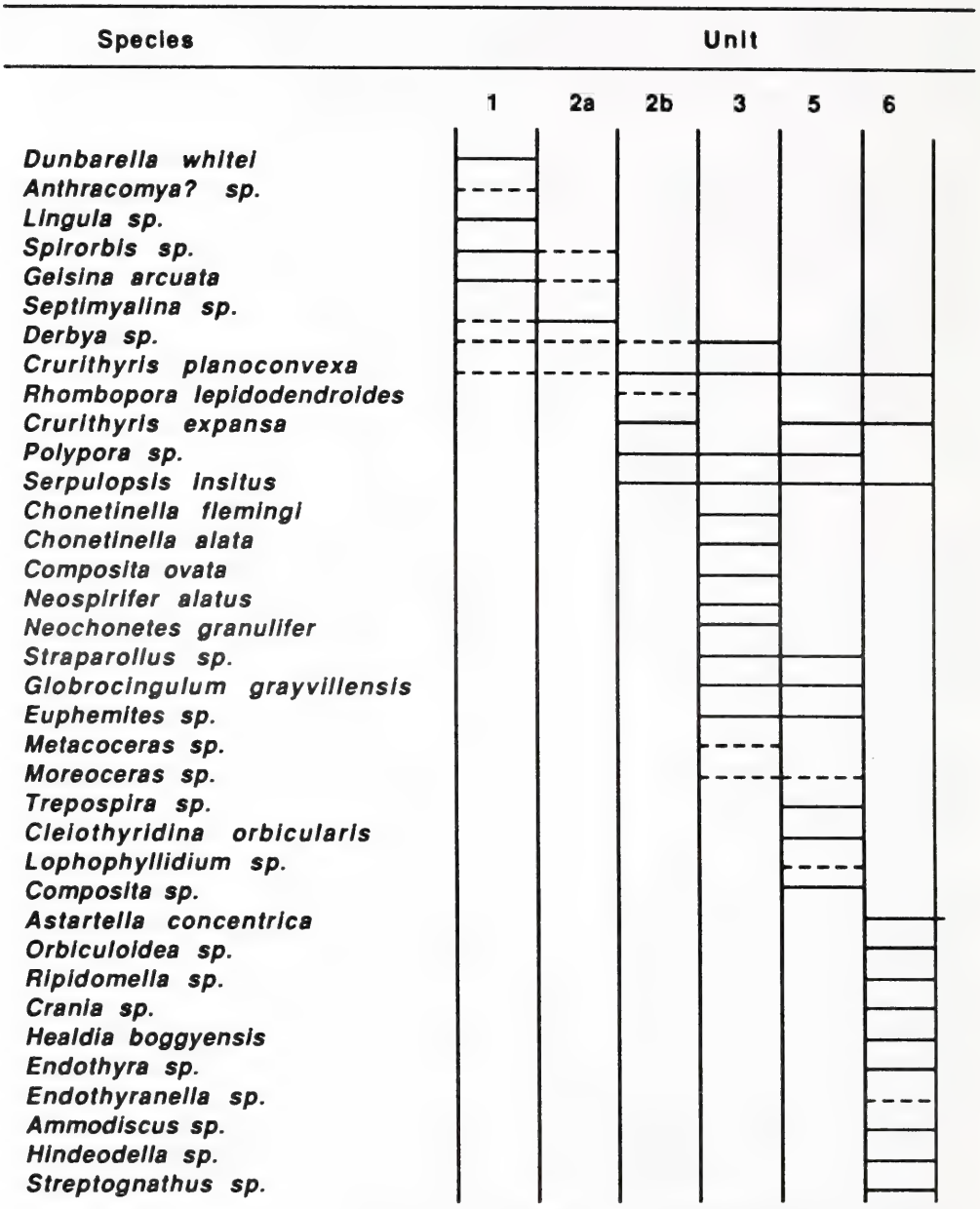

FIGURE 4. Fossils that occur in the rocks at Grayville and the lithologic units in which they occur (solid lines indicate common taxa and dashed lines indicate rare taxa).

The association also contains abundant, small intertwined tubes that are here assigned to *Serpulopsis insita* (White). *Serpulopsis* was originally considered to be the tubes of a serpulid worm, but was reassigned to the Foraminifera (6). Rocks in which it occurs in west-central Indiana are described as having "great numbers of *Serpulae* which fill the entire mass" (1). The fossil may have been an important sediment binding or trapping organism. *S. insita* is abundant in rocks above unit 2 at Grayville and in the Riverview Limestone at the Wabash cutoff.

Gastropod-Brachiopod Association.—This association is dominated by a diverse group of brachiopods. Associated are three species of gastropods, *Serpulopsis*, and rare orthoconic cephalopods. Epibenthonic suspension feeders dominate, but the gastropods were probably detritus feeders or scavengers (10). The high diversity in-

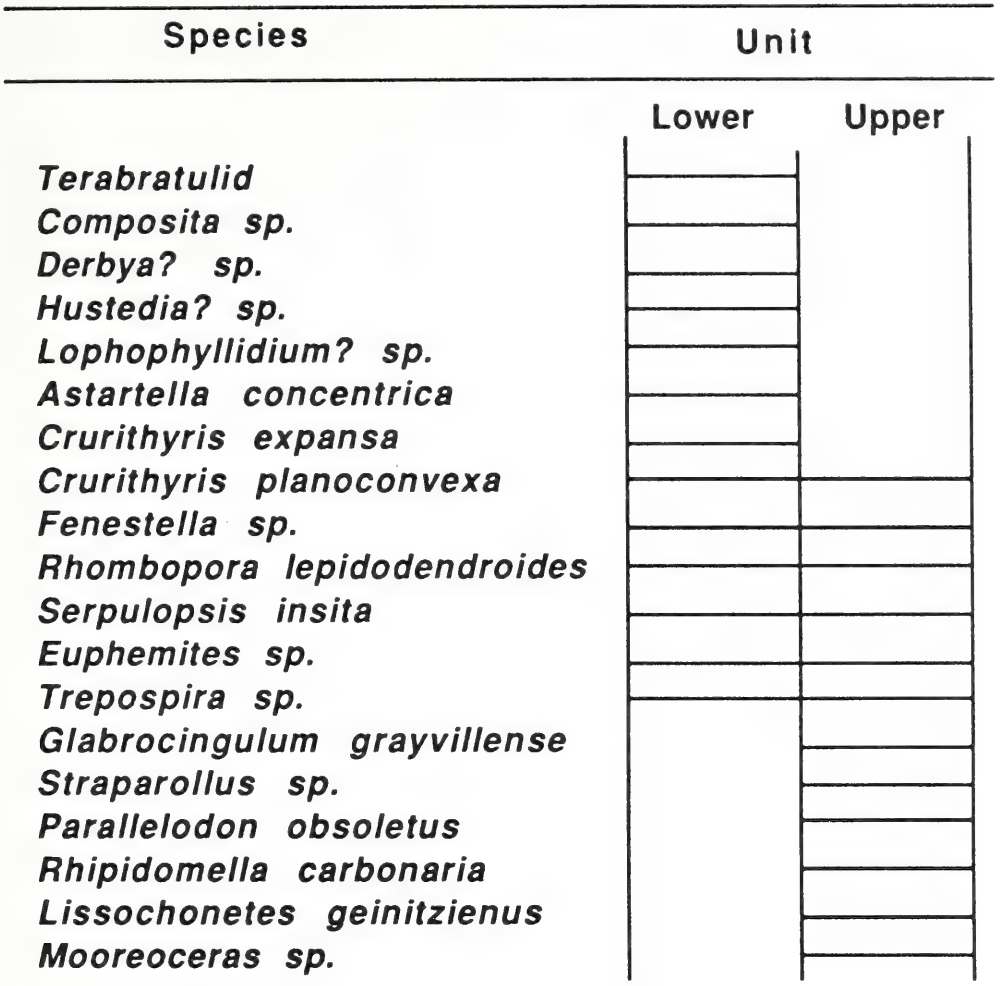

FIGURE 5. Fossils that occur in the rocks at the Wabash Cutoff and the lithologic units in which they occur (solid lines indicate common taxa and dashed lines indicate rare taxa).

dicates that the unit was deposited during a period of environmental stability. The association occurs in unit 3 at Grayville.

Gastropod Association.—This association is dominated by four species of gastropods. *Crurithyris* and *Serpulopsis* are abundant, and other brachiopods and bryozoan fragaments are rare. This association occurs in unit 5 at Grayville and in the upper part of the Riverview Limestone at the Wabash Cutoff.

This association was probably the result of marginal marine to slightly brackish water conditions. Unstable environmental conditions were probably responsible for both the lack of a diverse brachiopod component and the proliferation of *Crurithyris*. The gastropods were probably detritus feeders. They are most commonly considered to have prospered in marginal marine conditions during the Pennsylvanian Period. Slight differences between the two occurrences of the association exist (Fig. 4 and 5).

Foraminiferal Association.—This is the most diverse and equitable fossil association. Microfossils are an important element. Foraminifers are abundant, *Ammodiscus* being the most common. Conodonts are fairly common. With the exception of *Crurithyris* and *Serpulopsis* this association is dominated by species that do not

occur in other units. The abundance of foraminifers suggests more offshore, normal marine conditions (27), and increased diversity supports this interpretation. The lack of corals and fusulinids suggests that fully normal marine salinities were not attained. The Formainiferal association occurs in unit 6 at Grayville.

Brachiopod-Coral Association—This association is very similar to the Gastropod-Brachiopod association that occurs in Unit 3 at Grayville. The main difference between the two is the presence of the coral *Lophophyllidium* in this association. The coral may indicate that more normal marine salinities were present during deposition of this association. The brachiopod-coral association occurs in the lower part of the Riverview Limestone at the Wabash Cutoff, and most of the fossils here are large and nearly whole.

Paleoecologic Interpretation

Grayville

The lithologic sequence at Grayville, Illinois is the product of transgression of marine waters over a nonmarine, deltaic environment. Nonmarine deposition took place in clastic swamp areas in which the conchostracan *Pemphilimnadiopsis* lived. Distributary channels cut through this swamp and lense-like characteristics of overlying marine units suggest that transgression took place largely within distributary channels. Vertical changes in faunal associations are the product of community replacement rather than autogenic community succession. Replacement was due to lateral shifting of environments, and not to *in situ* replacement resulting from transgression (9).

The species richness of the associations in terms of number of common species occurring in each lithologic unit is shown in Figure 6. Those species that are represented by only one or two specimens are not included because they may have been transported. Species richness is taken here to be an indicator of faunal diversity. Diversity may be controlled by a number of factors. Physically unstable, resource rich environments generally support low diversity faunas, but stable, resource poor habitats should be dominated by diverse faunas (28).

Faunal diversity increases from Unit 2a through Unit 6, after an initial decrease from Unit 1 to Unit 2a. Diversity increase may have been a result of increasing salinity, distance from shore, or both (27). The occurrence of corals, foraminifers and bryozoans in the uppermost unit supports the conclusion that the locus of deposition became increasingly saline through time.

The *Dunbarella* association thrived in brackish water created by mixing of fresh and marine waters. Salinity fluctuation contributed to environmental instability. The very low salinities prevented many marine species from colonizing this habitat. As marine waters transgressed up distributary channels the locus of black shale deposition moved landward and the *Dunbarella* association was replaced by the *Septamyalina* association. The latter occurrence resembles modern oyster banks that grow on any firm substrate in tidal channels (29). Increasing diversity in higher associations indicates increasing environmental stability.

Wabash Cutoff

Data collected from the Wabash cutoff occurrence of the Riverview Limestone member are less meaningful than are those for the Grayville section. Fossils were more difficult to remove from the limestone and a consistent lithologic break within the limestone was not obvious so that reliable diversity-based trends were not observed.

Two associations occur in the limestone: the brachiopod-coral association in the lower part and the gastropod association in the upper part. The most important difference between the two associations is the presence of corals, bryozoans and a diverse

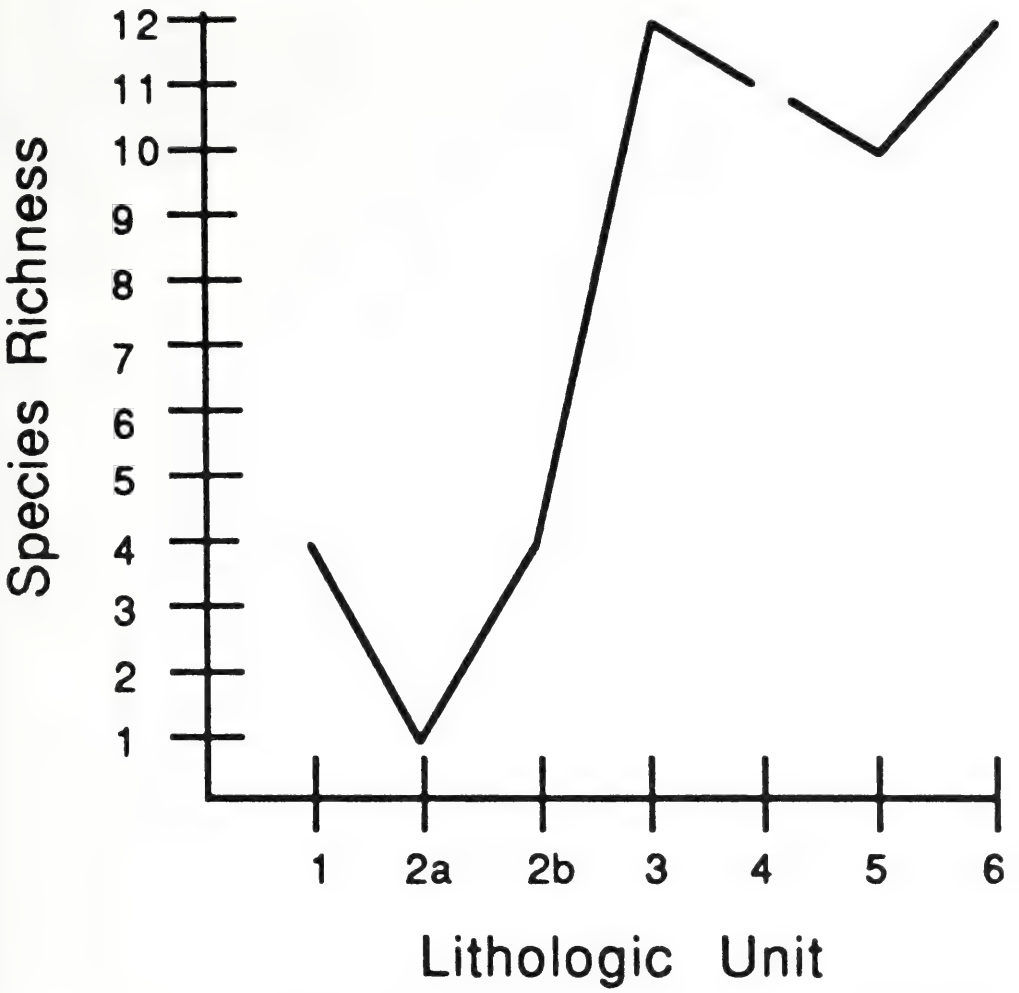

FIGURE 6. Graph showing diversity in terms of species richness (number of common species) plotted against stratigraphic position at Grayville.

brachiopod fauna in the lower association, and absence of most of these fossils in the upper. These organisms are generally regarded as being associated with fully normal marine conditions. The upper association is more restricted in habitat and components generally regarded as inhabiting normal marine waters are not present.

At the Wabash cutoff locality, the Riverview Limestone records a marine transgression. Transgressing marine waters encroached over a nonmarine coal swamp that is recorded by a thin coal bed that lies next below the limestone. The occurrence of normal marine fossils in the lower part of the limestone, just above the coals, indicates that transgression was rapid. No transitional brackish water or marginal marine fossils were found, as would be expected if transgressions was slow enough to allow preservation of replacement communities. Cataclysmic, possibly storm related, inundation of a coastal coal swamp may have been responsible for the sequence seen at the cutoff, but large fossil fragments in the lower association indicate that energy levels were neither high enough nor persistently strong enough to break up the fossils. An abrupt marine transgression with fully marine waters may have been responsible for the lower association, followed by downgrading of the environment and numerous storms, resulting in the low diversity and numerous shell fragments of the upper association.

Conclusions

Two of the four original New Harmony area collecting sites are still exposed. The other two apparently are covered by alluvium or filled in. Our collections from the two available sites yielded topotypes of three gastropod species that were described in early reports: *Euphemites blaneyanus, Euphemites vittatus*, and *Glabrocingulum (Glabrocingulum) grayvillensis*.

The marine rocks at both collecting sites were deposited during transgressional marine phases. Both lay above coal and clastics that were deposited in nonmarine environments. The two sites differ in style of marine transgression.

Deposition of the Grayville Limestone was slow, leaving a good record of fossil replacement through time. A brackish water association at the base was overlain by progressively more marine faunas. Slight changes in environmental stability affected the occurrence of specific fossils and the species richness of individual units.

Marine transgression that resulted in deposition of the Riverview Limestone at the Wabash cutoff was rapid. An association of normal marine fossils directly overlies coal, suggesting that marine inundations was sudden, possibly catastrophic. After marine inundation, the environment was downgraded and a relict community persisted for a short time. Nonmarine deltaic siltstone and sandstones replaced the shallow marine environments.

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APPENDIX 1

List of original species names and localities for species that were originally described from the New Harmony area.

McChesney, 1860

Rhynchonella algeri	Big Creek
Edmondia concentrica	Big Creek
Zeacrinus mucrospinus	Mouth of Rush Creek
Leda oweni	Mouth of Rush Creek
Bellerophon vittattus	Mouth of Rush Creek; Grayville
Orthoceras rushensis	Mouth of Rush Creek
Rhynchonella eatoniaeformis	Grayville
Nautilus quadrangulus	Grayville
Nautilus nodocarinatus	Grayville
Rhynochonella explanata	"New Harmony"
Leda gibbosa	"Five miles below New Harmony"

Norwood and Pratten, 1855a

Pleurotomaria grayvillensis	Mouth of Rush Creek; Grayville
Bellerophon monfortianus	"Five miles below New Harmony"
Loxonema hallii	"Five miles below New Harmony"
Natica ventrica	"One mile south of New Harmony"

Norwood and Pratten, 1855b

Productus splendens	Big Creek
Productus portlockianus	Grayville
Productus clavus	Grayville
Productus wabashensis	"New Harmony"

Meek and Worthen, 1860

Solemya radiata	Grayville
Platystoma? tumida	Grayville
Cyrtoceras curtum	Grayville
Pleurophorus? angulatus	Wabash cutoff
Conocardium obliquum	Wabash cutoff
Schizodus curtus	Wabash cutoff

Hall, 1858

Edmondia radiata	Grayville

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Engineering Characteristics of Lacustrine Deposits Associated with Coal Strip Mining, Southwest Indiana

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Introduction

During the Pleistocene, hundreds of valleys in southwest Indiana were occupied by meltwater lakes. Lake beds were formed in tributary valleys of heavily aggraded glacial sluiceways and today they generally extend beyond the ice-contact boundaries. Most of the lake beds are now veneered with a blanket of loess which makes it more complicated to obtain accurate surface maps depicting these lacustrine deposits.

The lake beds consist of unconslidated, interfingered sediments and are directly associated with outwash, dune sand, loess and sometimes glacial till. The lacustrine sediments themselves consist primarily of silt and clay with thin layers of sands, organics, and marls. They were deposited in quiet waters adjacent to upland areas composed primarily of Pennsylvanian-aged bedrock. The overall stratigraphy of the system is extremely complex.

Many of these lacustrine deposits overly the strippable coal reserves of the state. Owing to their low shear strengths, these deposits create problems for coal strip mining operations such as highwall excavation and cast over spoil piles. The deposits are frequently saturated and because of their very low permeabilities and complicated stratigraphies, they cannot be dewatered prior to excavation.

Previous research performed at Purdue University by Greengold (1981a, 1981b) and Oschman (1984) examined the engineering problems of lacustrine materials associated with coal strip mining areas in Indiana and Illinois, respectively.

Lacustrine deposits were encountered in the construction of Interstate 164 which will be a highway bypass on the east side of Evansville. Exploration and testing data obtained from this highway project (Atlas Soils Inc., 1985) provided additional engineering data on these deposits.

A study involving lacustrine deposits and coal strip mining in southwest Indiana is currently underway at Purdue University to determine in more detail the nature and engineering characteristics of these deposits.

This paper examines the extent and origin of these lacustrine deposits and provides engineering characteristics for these problematic soils.

Origin and Extent of Lake Beds

Major streams that drained southwest Indiana carried such great loads of sediment during glacial times that their valleys became heavily aggraded. This resulted in blockage and ponding of hundreds of tributary streams. Thick deposits of lacustrine silts and clays filled these valleys.

The majority of the lake beds lie in the Wabash Lowland physiographic division. Malott (1922) characterized this landform unit as alluvial in origin and emphasized the prevalence of aggraded valleys. Glacial till partially covers the Wabash Lowland and is underlain by lacustrine, outwash and alluvial sediments. Upland areas are undulating to rolling plains covered by eolian sediments that generally thin to the east from the Wabash River valley train source (Schneider, 1966). The Wabash

Lowland developed from valley-widening caused by pre-glacial erosion of weak Pennsylvanian limestones, sandstones, and shales.

Pennsylvanian strata comprise the Sullivan Lowland bedrock physiographic division. Topography on the bedrock surface is generally much more irregular than is the present topography of the land surface, however, from a regional view point, the relief is smooth and gentle. Bedrock elevations typically occur below 500 feet elevation. Bedrock valleys trend southwest toward the Wabash and Ohio Rivers.

Lacustrine deposits of this study area are only one of the facies of the Atherton Formation which spans the entire Pleistocene epoch in Indiana. Wayne (1963) assigned this name to a group of unconsolidated sediments that resulted from glacial action, but were generally deposited beyond the glacial limits by the action of meltwater or wind. He identified four distinct facies within the Formation. They are: 1) outwash facies, 2) lacustrine facies, 3) dune facies, 4) loess facies. The sediments were primarily derived from outwash, and they interfinger with each other. Figure 1 schematically illustrates the facies of the Atherton Formation.

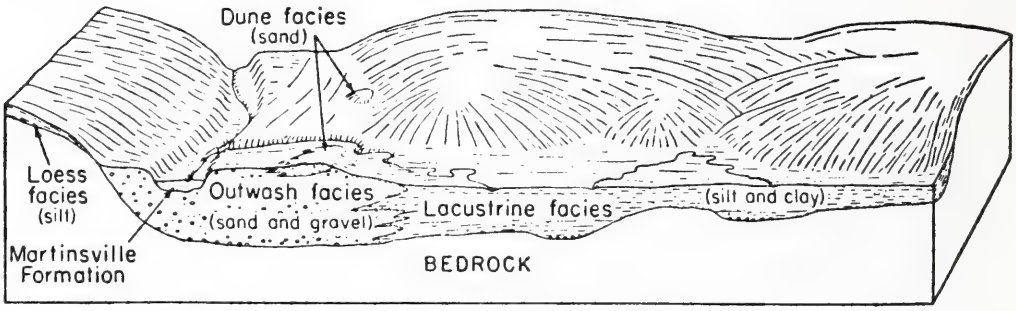

FIGURE 1. Schematic diagram showing the facies relationships of the Atherton Formation (Wayne, 1963).

Figure 2 represents the distribution of glacial lacustrine deposits in southwest Indiana. Note the dendritic patterns of the deposits indicating that the lakes occupied pre-existing valleys. Because many of these deposits are covered with loess, and a minimum mapping thickness of approximately six feet was used, the unit boundaries should be considered only approximate.

1981 Purdue University Study

In 1981 a study was completed at Purdue University (Greengold, 1981a) involving coal strip mining and lake bed clays. The study area is located near Petersburg, Indiana, (Pike County) approximately 30 miles north of Evansville. Figure 3 gives the site location.

For this coal mine area, subsurface investigations indicated the topography varies in excess of 50 feet in elevation for a site of about 4 square miles. The land surface of the site prior to strip mining also had a relief of nearly 100 feet. Because of this combination the thickness of the unconsolidated materials varied from 0 to 105 feet.

Unconsolidated materials consist of loess, glacial-lacustrine sands, silts and clays; and glacial till which collectively yield an extreme range in soil properties. The lake beds were deposited in glacial Lake Patoka which was in excess of 100 square miles in area.

Stratigraphy is dominated by organic-bearing lacustrine clays and loose, fine to medium, saturated sands. Sands and clays range from small pockets to others greater than 50 feet thick and hundreds of feet across. There are two lake bed deposits

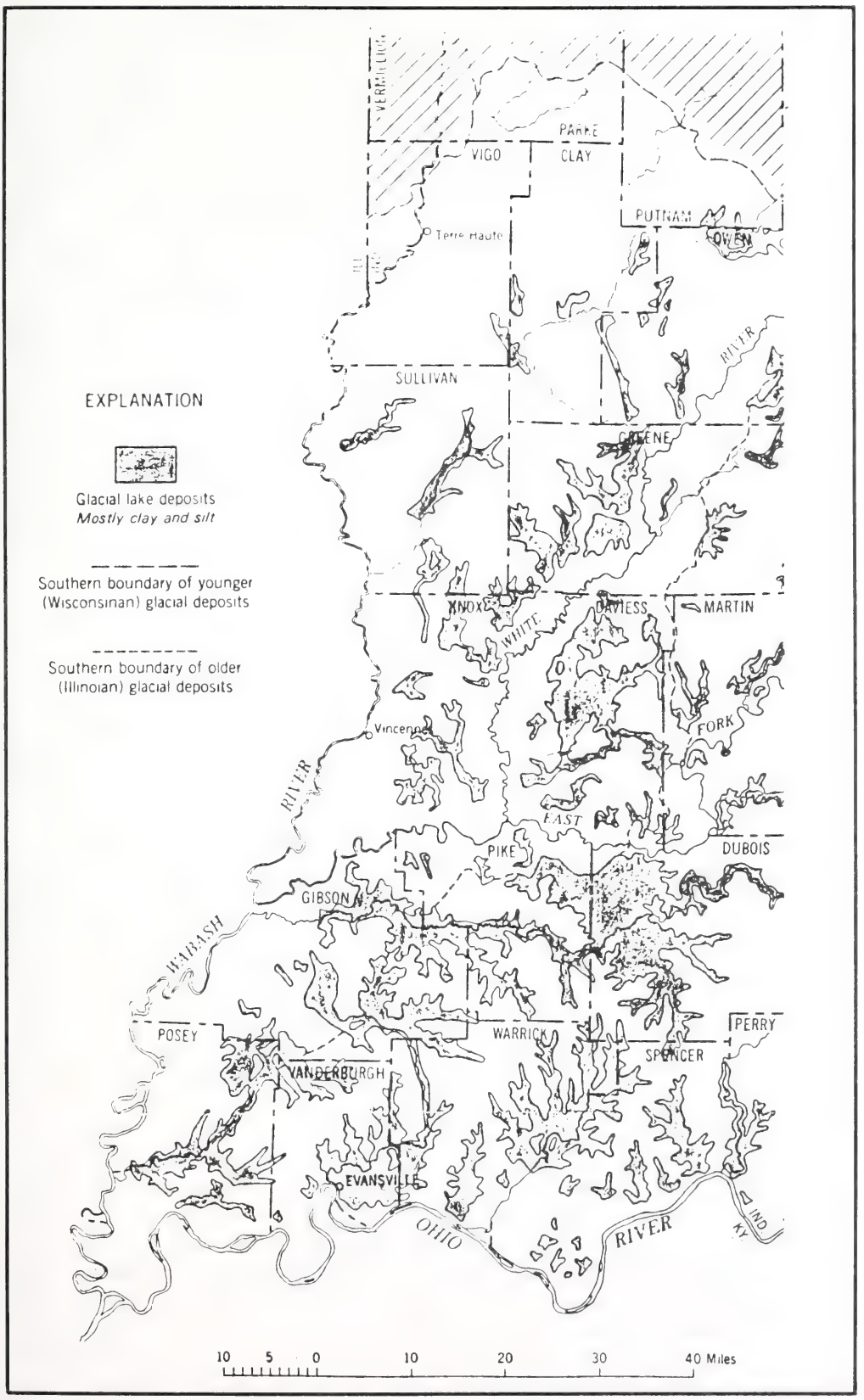

FIGURE 2. Map showing the distribution of glacial lacustrine deposits in southwestern Indiana (from Gray, 1971).

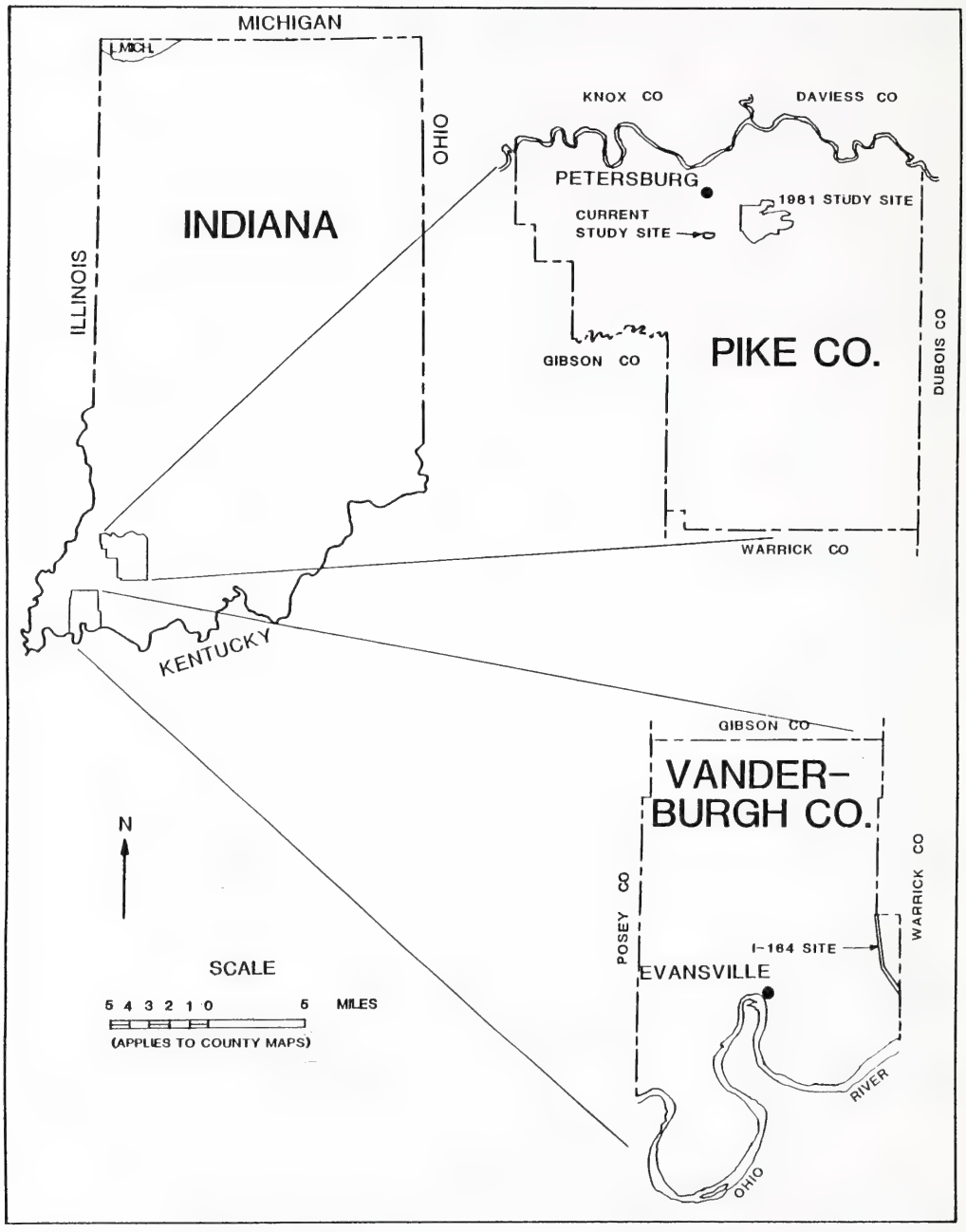

FIGURE 3. Site locations map.

on the four-square mile site. The northern unit with an areal extent of about 1/4 of a square mile is Wisconsin in age. The southern unit is of Illinoian age and is about one square mile in extent. Bedrock is the Pennsylvanian-aged Dugger Formation, here a gray sandy shale interbedded with yellow and gray sandstone and some limestone. The coal seam being mined is the Indiana Number V seam or Springfield Coal which is about 6 feet thick at this location.

Samples were obtained in borings by standard penetration tests and by Shelby tube sampling. Split spoon samples were taken at 5 foot intervals. Twelve borings were accomplished. Two layers of unconsolidated materials were indicated; the upper layer 18 to 23 feet thick showed N values ranging from 8 and 22 blows per foot.

This layer presumably included loess, lake silts, alluvium and glacial till. The lower layer includes glacial till, plus gray lacustrine clays and sands. Blow counts in the lower unit ranged from zero in the loose saturated sands to 91 blows per foot in the very stiff glacial till.

Shear strengths for the gray lacustrine clay ranged from 0.08 to 1.37 tons/ft^2 with an average of 0.6, based on 15 unconfined compression tests and 16 shear vane tests. The loess had a range of shear strength from 0.08 to 1.44 tons/ft^2 and an average of 0.7, based on four unconfined tests and four vane tests. The yellow clays or "lake silts" averaged 0.08 tons/ft^2 for two shear vane tests of 0.04 and 0.11 tons/ft^2. In a previous study of the coal mine area, values ranging from 0.42 to 2.03 were obtained for the silty yellow clays. In that earlier study shear strengths of the glacial till ranged from 2.51 to 5.25 tons/ft^2 with dry unit weights between 94 and 101 lbs/ft^3.

In the study by Greengold (1981a) the sensitivity of the gray clay averaged 1.7 based on 15 shear vane tests. Values ranged from 1.2 to 2.5. Clays with sensitivities < 2 are considered to be insensitive (Bowles, 1979, p. 377). The loess has an average sensitivity of 1.5 (2 tests) whereas the lake silts averaged 1.6 (2 tests).

Units weights for the loess (in situ or wet density) ranged from 106 to 133 psf, averaging 124 pcf for four samples. The lake silts (calcareous, pebbly yellow clays) ranged from 120 to 135 pcf, averaging 125 pcf for two samples. The gray lake clays varied from 113 to 144 pcf with an average of 139 pcf for 13 samples.

Water content of the gray clay ranged from 17 to 46% with an average of about 26% for 29 samples. In a general way shear strength decreased with increasing water content but there was not a strong correlation between them.

Again with respect to the gray lacustrine clay the shear strength did not increase with depth (or with increasing overburden pressure). Instead the lowest values of shear strength were found near the base of the gray lacustrine clay. This indicates a potential slope stability problem when these materials are excavated during strip mining.

For this strip mine in Pike County, Indiana slope stability in the spoil piles formed by the large drag line is also a concern. The unconsolidated material ranges from 0 to 105 feet thick as stated previously and the rock overburden above the coal seam ranges upward to 160 feet thick. Where soils comprise more than 40% of the total overburden section, spoil pile instabilities arise as rock volume is insufficient to retain the soil. The rock spoil is piled into two parallel rows with the soil placed in the V-notch between them. When the soil comprises more than 40% of the total overburden it overflows the V-notch volume. In such cases, the extra soil must be stripped away prior to the dragline operation and moved to the high wall side of the cut using large trucks.

In the study by Greengold (1981a) several recommendations were made to reduce slope stability problems in the highwall. These included: dewatering sands where applicable, benching in thick soils to reduce slope failures and reorienting the pit to prevent the concentration of soft material in certain sections of the excavation.

Interstate Highway I-164 Study

Interstate Highway 164 is currently under construction east of Evansville, Indiana. When completed it will extend for about 13 miles connecting Indiana State Road 66 to Interstate 64. With almost a north-south alignment, it will serve as a bypass around the east side of Evansville (Atlas Soils Report, 1985).

I-164 is located close to the north-south boundary between Warrick County on the east and Vanderburgh County on the west. These two counties are immediately north of the Ohio River in southwestern Indiana (Figure 3).

The site lies south of the maximum extent of Illinoian glacial ice in the unglaciated

portion of Indiana. Glacial fluvial deposits in the form of lake bed clays and sandy outwash occur in southern Vanderburgh and Warrick Counties. In the extreme southern part of the counties near the Ohio River, alluvial sand prevails as stream deposits and adjacent terraces. Lacustrine deposits are found northward for several miles until residual soils formed by the weathering of Pennsylvanian shales and siltstones prevail. The lacustrine deposits of gray clay and sand are found up to 50 feet thick in eastern Vanderburgh County.

To obtain additional details on the engineering properties of lacustrine clays, a 4.7 mile section of the design plans for I-164 were evaluated. For this section of road, 37 road centerline borings were taken and 36 structure borings were completed. The road borings ranged in depth from 6 to 36 feet deep whereas the structure borings, located at bridge overpass structures, were typically 30 to 80 feet deep. Many of these extended to bedrock (West, 1987).

In this area the upper 10 feet or so was typically a brown silty loam, i.e. loess. This wind deposited silty material is prevalent on the eastern side of the Wabash River in Indiana. The gray silty clay, lacustrine materials occurred below the loess. The gray clays had standard penetration values (N values) ranging from 7 to 30 blows per foot with typical values about 15 to 20. Moisture contents ranged from 16 to 50% with most values around 26%. The plasticity index for the gray clays was about 12, with liquid limits and plastic limits about 32 and 20, respectively. Wet unit weights ranged from 117 to 132 lb/ft^3 with natural moisture contents for these samples about 25%.

Unconfined compression tests had been run on 17 soil samples. Shear strengths ranged from .066 to 1.50 $tons/ft^2$ with an average of 0.56 $tons/ft^2$. Rimac values had been made on the split spoon samples following drilling. For 374 samples tests an average of 0.935 T/ft^2 was obatined for these tests. Comparing the two answers, 0.935 is 1.67 times greater than the 0.56 value obtained from unconfined compression tests. The Rimac values tend to be high for such comparisons.

Eleven triaxial compression tests had been run on the samples. The cohesion values ranged from .25 to 1.3 $tons/ft^2$ and the ϕ angles from 0 to 3.8 degrees.

Current Research

A study is presently underway at Purdue University involving lacustrine clays and coal strip mining in southwest Indiana. The Prides Creek-East Pit owned by Solar Sources, Inc. is located in Pike County, two miles south of Petersburg, Indiana (Figure 3). A thick lacustrine deposit was encountered by the coal company during strip mining operations at the western boundary of their property. This is an on going research project supported by the U.S. Bureau of Mines, through the Indiana Mining and Minerals Resources Research Institute.

Elevation of the bedrock surface at the site varies more than 70 feet over a distance of approximately one half mile from east to west. The original landsurface across the same location varied approximately 30 feet. Thickness of the unconsolidated materials at the site ranged from approximately 3 to 60 feet. These isolated lake bed deposits had an areal extent of about seven square miles.

Unconsolidated deposits consist of loess, organic bearing to rich, highly calcareous silts, clays, and some sands. Glacial till was not encountered at the site. Bedrock consists of the Pennsylvanian Dugger Formation. Coals mined at the site are the Bucktown (Coal Vb) and Springfield (Coal V) coal members of the Dugger and Petersburg Formations, respectively.

A boring involving continuous split spoon sampling was made through the lacustrine section to bedrock in the summer, 1986. Upper and lower portions of each split spoon sample were collected. A stratigraphic section for the boring was developed

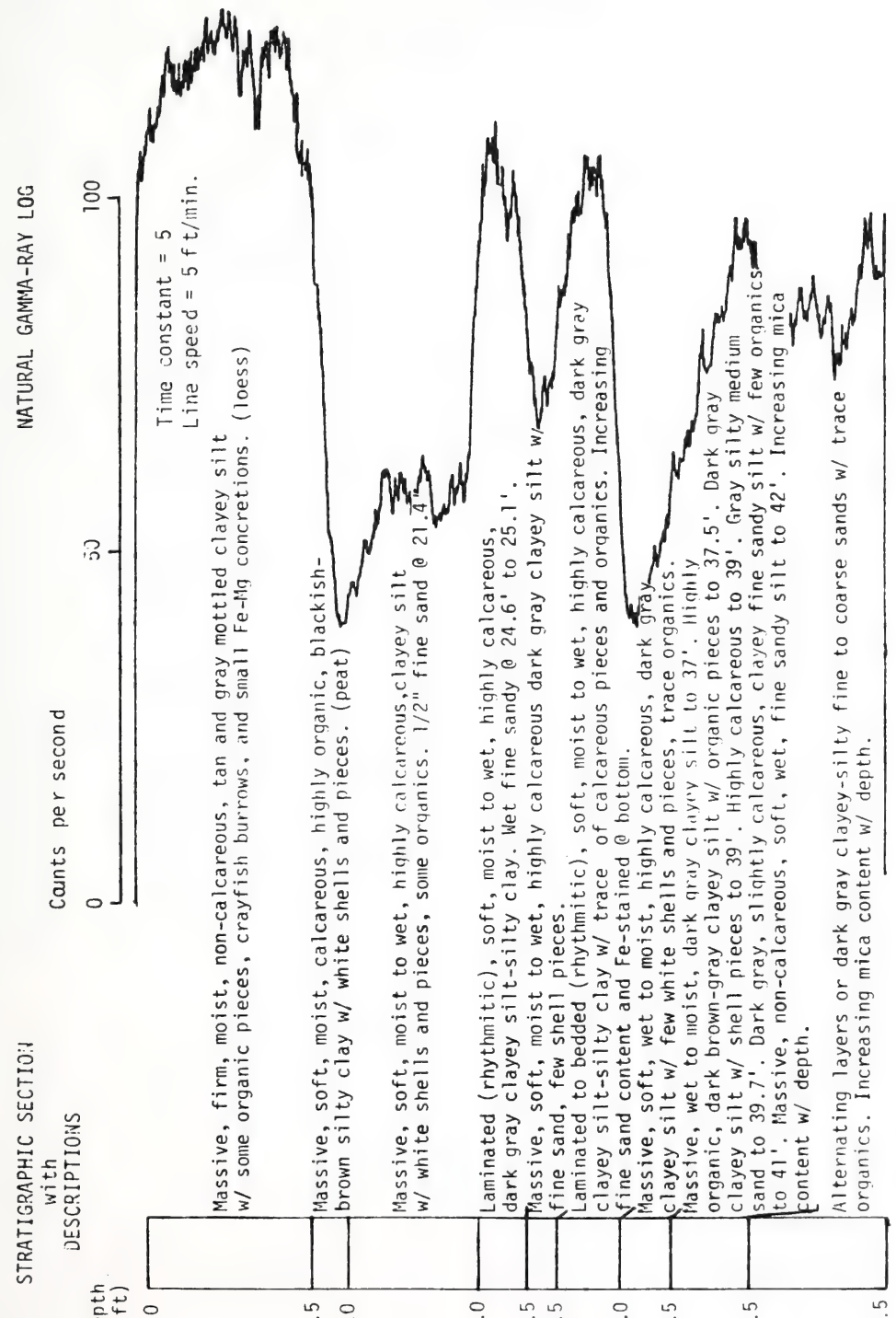
STRATIGRAPHIC SECTION
with
DESCRIPTIONS
NATURAL GAMMA-RAY LOG
Counts per second
0
50
100
Time constant = 5
Line speed = 5 ft/min.
Depth (ft)
0.0
Massive, firm, moist, non-calcareous, tan and gray mottled clayey silt w/ some organic pieces, crayfish burrows, and small Fe-Mg concretions. (loess)
11.5
Massive, soft, moist, calcareous, highly organic, blackish-brown silty clay w/ white shells and pieces. (peat)
14.0
Massive, soft, moist to wet, highly calcareous, clayey silt w/ white shells and pieces, some organics. 1/2" fine sand @ 21.4'
23.0
Laminated (rhythmitic), soft, moist to wet, highly calcareous, dark gray clayey silt-silty clay. Wet fine sandy @ 24.6' to 25.1'.
26.5
Massive, soft, moist to wet, highly calcareous dark gray clayey silt w/ fine sand, few shell pieces.
28.5
Laminated to bedded (rhythmitic), soft, moist to wet, highly calcareous, dark gray clayey silt-silty clay w/ trace of calcareous pieces and organics. Increasing fine sand content and Fe-stained @ bottom.
33.0
Massive, soft, wet to moist, highly calcareous, dark gray clayey silt w/ few white shells and pieces, trace organics.
36.5
Massive, wet to moist, dark gray clayey silt to 37'. Highly organic, dark brown-gray clayey silt w/ organic pieces to 37.5'. Dark gray clayey silt w/ shell pieces to 39'. Highly calcareous to 39'. Gray silty medium sand to 39.7'. Dark gray, slightly calcareous, clayey fine sandy silt w/ few organics to 41'. Massive, non-calcareous, soft, wet, fine sandy silt to 42'. Increasing mica content w/ depth.
42.5
Alternating layers or dark gray clayey-silty fine to coarse sands w/ trace organics. Increasing mica content w/ depth.
51.5

FIGURE 4. Stratigraphic section and natural gamma-ray log of boring at the Prides Creek site.

from the drilling logs and samples which was subsequently compared to the natural gamma-ray log of the boring. The gamma-log provides a signature for the deposit. Figure 4 presents the gamma-log signature and a descriptive stratigraphic section of the deposit. The boundaries shown in the section are somewhat penetration values (N) of the deposit ranged from 5 to 9 for the loess and from 2 to 5 for the lacustrine silts and clays.

In table 1, results are presented for laboratory analysis completed on bag samples collected from the exposed face at the Prides Creek site.

Adjacent to the split spoon boring, a second boring was made to collect undisturbed samples using pushed Shelby tubes. Unit weights, natural moisture contents, and unconfined compressive strength tests were performed on these samples.

The average natural unit weight for the loess was 121.5 pcf with an average natural moisture content of 24%. The shear strength ranged from 0.32 to 0.56 ton/ft^2 and averaged 0.42 ton/ft^2.

The lacustrine silts and clays occur below the 11.5 foot thick surficial loess layer. Their natural unit weights ranged from 99 to 122 pcf. The average of eleven determinations was 114 pcf. Unconfined compression tests were run on 20 samples. Shear strengths ranged from 0.16 to 0.60 ton/ft^2. The average was 0.40 ton/ft^2. Natural moisture content ranged from 21 to 67% with an average of 41.1%, based on 31 determinations. The results of these tests are summarized in Table 2.

It is clear that the strength of this deposit is quite low. The lacustrine silts and clays are reasonably plastic with plasticity indices ranging from 4 to 51. The clay content ranged as high as 52%. The lacustrine soils are designated as ML, CL, CH, and OH according to the Unified Soil Classification. As in the Greengold (1981a) study, there was no strong correlation between water content and shear strength.

X-ray diffraction analyses were run on the clay size fraction of the lacustrine deposit. The analyses showed that the lacustrine deposit contains the following clay minerals; montmorillinite and possibly vermiculite, illite, chlorite, and kaolinite. The mean approximate percentages of the clay minerals are: Expandables—53.8%, Illite—25.8%, Kaolinite + chlorite—20.4%. The high percentage of the expandable clay minerals underscores the stability problems encountered at the site. As the lacustrine materials are excavated, the highwall face is open and exposed to repeated wetting and drying cycles. Studies have shown that the shear strength of expansive clays containing montmorillinite decreases as a result of wetting and rewetting (Gibbs et al., 1960).

Comparison to Test Results

A comparison can be made for the gray lacustrine clays for the three sites discussed above. This is summarized in Table 2.

From this comparison it is evident that the lacustrine deposits of the current study area are weaker than those of the other two sites. In the current study area the soils are more plastic and have higher natural moisture contents. Also, they contain a large percentage of clay minerals. X-ray analyses indicate that greater than 50% of the clay minerals are of the expandable types. The characteristics of the other two sites are perhaps more typical of lake bed deposits for larger depositional areas.

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TABLE 1. Soil Samples Results, Prides Creek-East Pit

Description	Approx. Depth (ft)	N value blows/ft	Liquid Limit (%)	Plastic Limit (%)	Plasticity Index	% Silt (<.004mm)	% Clay (>.004mm)	Unified Soil Classification
Gray w/tan mottling slightly clayey silt with organics	6	6	28	18	10	71	25	CL
Dark gray soft moist silty clay w/some small calcareous pieces	13	3	88	37	51	43	43	OH
Dark gray soft moist silty clay w/small white calcareous pieces	17	2	50	36	14	72	17	MH
Dark gray moist to wet silty clay w/ a few small calcareous pieces	22	2	51	26	25	76	7	CH
Dark gray wet soft clay to silty clay w/ a few small calcareous pieces	26	4	32	28	4	83	9	ML
Dark gray clayey silt w/ a few small calcareous pieces	30	5	24	17	7	72	23	CL
Dark gray moist to wet clayey silt w/ trace of small calcareous pieces and trace organics	30.5	2	33	16	17	48	52	CL
Dark gray moist clay w/ a few small calcareous pieces	33	3	39	30	9	78	11	ML
Dark gray soft silty clay	37	2	35	27	8	77	11	ML

TABLE 2. Comparison of results, three locations of lacustrine clay deposits.

Study Designation	N value Blows/ft	Shear Strength Range T/ft²	Average Shear Strength T/ft²	w% Range	w% Average	PI Range	PI Average	Sensitivity		Natural Unit Wt.		Dry Unit Wt.	
								Range	Avg.	Range pcf	Avg. pcf	Range pcf	Avg. pcf
1981 Study	8-22	0.08-1.37	0.6	16-53	26.4	—	—	1.2-2.6	1.7	106-144	129	76-122	103
Current Study	3-5	0.16-0.60	0.4	21-67	41.1	4-51	17	—	—	99-122	114	59-100	82
I-164 Study	7-30	0.07-1.50	0.56	16-50	25.8	7-21	12	—	—	117-132	125	88-109	99

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Determination of the 10,000 mg/1 TDS Surface Within the Bedrock Aquifers of Indiana

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Introduction

Evaluation of the total dissolved solids (TDS) content of bedrock aquifers in Indiana indicates that the 10,000 mg/1 (ppm) TDS interface is a regionally mappable aquifer parameter. The position of this surface where the salinity of the ground water equals 10,000 mg/1 TDS is controlled by the hydrologic framework of the region, which includes the regional and localized geologic structure, the lateral and vertical permeability distribution, and the patterns of ground water flow under the influence of hydrologic boundary conditions.

Although water that contains 10,000 mg/1 TDS is considered brackish (1), this concentration of total dissolved solids was established by the U.S. Environmental Protection Agency as a maximum for potentially potable water. Determination of the 10,000 mg/1 TDS surface is therefore important to petroleum-producing industries, steel-manufacturing industries, and users of potable water from deep bedrock aquifers.

Salinity Determination

In order that the general patterns of salinity distribution in deep bedrock aquifers throughout the state be established, approximately three hundred records of subsurface borings were examined. Most of these records were composed of geophysical logs, drill stem test results, driller's records, and rock samples (drill cuttings). Much data were from oil and gas investigations. Additional data were derived from stratigraphic test holes, mineral exploration borings, and underground storage operations.

Although in reality the surface is probably a transition zone between brackish and saline water (1), for mapping purposes the position within the stratigraphic column where the salinity of the formation water is equal to 10,000 mg/1 TDS is considered to be a point on this surface. Three methods were utilized to determine the hydrostratigraphic position of the 10,000 mg/1 TDS surface: direct salinity measurement, interpretation of geophysical logs, and qualitative analysis of formation water.

The most reliable means of determining the TDS content of a given hydrostratigraphic unit is direct measurement coupled with laboratory analysis (2). Water samples from deep aquifers may be recovered from drill stem tests, swab and pumping tests, and cable-tool bailers. Samples analyzed in the laboratory yield a more complete and accurate determination of anion and cation concentrations responsible for TDS values. Documentation of the various major ionic components also allows for greater confidence in the determination of TDS levels. On-site electrical conductance tests provide a check on the laboratory analyses. A laboratory gravimetric determination of the bulk TDS value is possible but lacks the accuracy of a major ion analysis.

The second method of determining the salinity of these waters is by interpretive calculation from geophysical logs. Log calculations entail a series of assumptions, some of which are practically and economically documentable. The assumptions are:

NaCl is the sole source of dissolved solids, the temperature of the formation can be accurately established, and the petrophysical properties of porosity, permeability, and pore throat tortuosity and cementation are known. There were three different methods of log calculation used in this study: 1) the static spontaneous potential (SSP) method, 2) the resistivity-porosity (R_t-ϕ) method, and 3) the "quick-look" method. The applications of the first two methods to salinity determination of subsurface brines in the Indiana part of the Illinois Basin were investigated by Geoscience Research Associates and Purdue University (3).

The static spontaneous potential (SSP) method is the most widely utilized and possibly the most accurate of the calculation methods (4). The basic relationship is:

$$R_w = R_{mf}{}^{10^{-T_a}}$$

where: R_w = resistivity of the formation water
R_{mf} = resistivity of the mud filtrate
T_a = SP/.11(460 + T) = coefficient proportional to the absolute temperature
SP = deflection of spontaneous potential log
T = temperature in °F

The SSP method is applicable only where a thick porous sand section lies within a shale (i.e. a clean sand/shale sequence). The method cannot be applied in a massive carbonate section where there is minimal SP deflection. The log must be run in a well drilled with freshwater-based mud. A SP/R_{mf}/temperature nomograph is commonly utilized to obtain a value for R_w (5). The R_w is then converted to the equivalent concentration of Cl^- in a temperature dependent chart (5).

The second means of log calculation, termed the resistivity-porosity (Rt-ϕ) method (5), is often used in thick massive carbonate sections when no SP curve is available. The method is also applicable for use in sections that contain thin discontinuous porosity breaks. The basic relationship is:

$$R_w = R_t/F \text{ and } F = a/\phi^m$$

where: F = formation factor
R = resistivity of the formation
a = empirically derived tortuosity coefficient
ϕ = porosity
m = empirically derived cementation exponent

F is determined most commonly by using the log-derived value for porosity and applying assumed values for cementation and pore-throat geometry based on lithologic interpretations. Actual values for a and m must be derived from laboratory analysis of cores. When values are not determined by laboratory analysis, values of 1 and 2.15 are usually assigned to these variables. Although a value for ϕ can be obtained from geophysical logs, there remains some question because of lithologic effects. As with the SSP method, a nomograph is used to derive a value for F from log-measured ϕ values, which is then divided into a log-measured value for R_t. R_w is again converted to equivalent Cl^- concentration in a temperature dependent chart.

The final log calculation method, the "quick-look," is a semi-quantiative method used when neither of the two previously described methods can be utilized but a resistivity log with deep (R_t) and shallow (R_{xo}) curves is available. This method basically compares the resistivity of the drilling mud with the resistivity of the formation water (4). From this comparison, an approximate value of R_w is obtained and converted to equivalent Cl^- concentration as in the first two methods. The relationship is:

$$R_w = R_{mf} (R_t/R_{xo})$$

where: R_{xo} = resistivity of the formation completely saturated with mud filtrate

Within the study area, the predominant type of log calculation utilized was the SSP method. Because some deep tests in northern Indiana lacked SP curves or contained the 10,000 mg/1 TDS interface within the Knox corbonate section, the Rt-ϕ method had to be used. The "quick-look" method was used most often in older tests in northern and eastern Indiana. All three methods were utilized in wells that had analyzed recovered fluids to cross-check the reported TDS values.

The third means of salinity determination is qualitative analysis of produced waters. If data are not available from laboratory-analyzed water samples or geophysical logs, an on-site comparison of recovered waters with waters of known salinity concentration is often helpful. The waters are qualitatively described in reference to taste, smell, and appearance. Comparison of this type of data in areas of low sample coverage can be meaningful for establishing basic salinity distribution in the rock column.

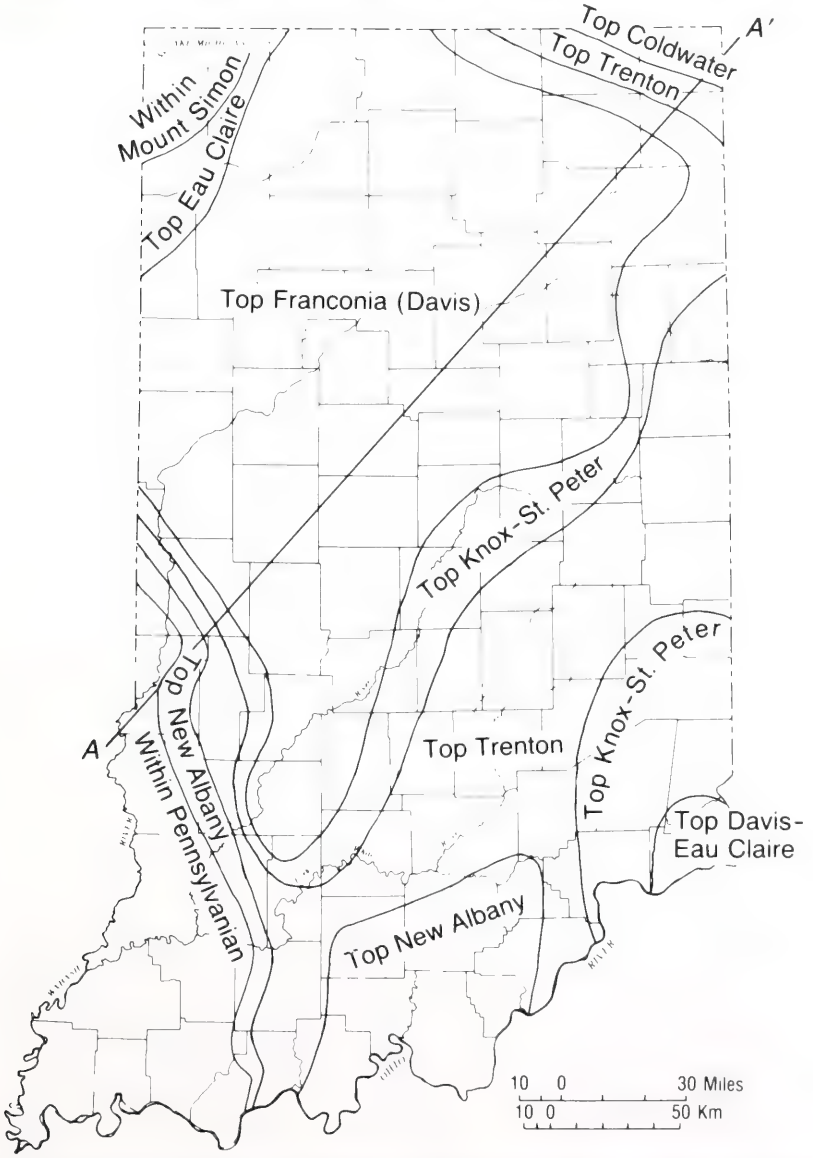

FIGURE 1. Map of Indiana showing hydrostratigraphic units in which the 10,000 mg/1 TDS surface is located. A-A' is the line of cross section shown in Figure 4.

Mapping of the 10,000 mg/1 TDS Surface

Data derived from the various sources of formation water salinities were combined to form a map of Indiana showing the hydrostratigraphic units in which the 10,000 mg/1 TDS surface lies (Figure 1). The hydrostratigraphic units identified in this study were derived by dividing the lithostratigraphic column into aquifers, intermediate-quality aquifers, and aquitards or confining units (Figure 2). The 10,000 mg/1 TDS surface was designated as the top of the uppermost units that contained waters of greater than 10,000 mg/1 TDS. If the aquifer that contained these higher salinity waters was overlain by a non-reservoir rock unit of very low permeability (such as the Franconia Formation or the New Albany Shale), the surface was positioned at the top of the overlying impermeable unit. Although some units are of poor aquifer quality (such as the Ancell and Black River Groups), small discontinuous porosity breaks within them can contain water of less than 10,000 mg/1 TDS and are therefore classified as within the potentially potable ground water system. For these units, the 10,000 mg/1 TDS surface is placed at the base of the intermediate-quality aquifer or therefore at the top of the underlying aquifer bearing saline water (e.g. the Knox-St. Peter aquifer) (Figure 2).

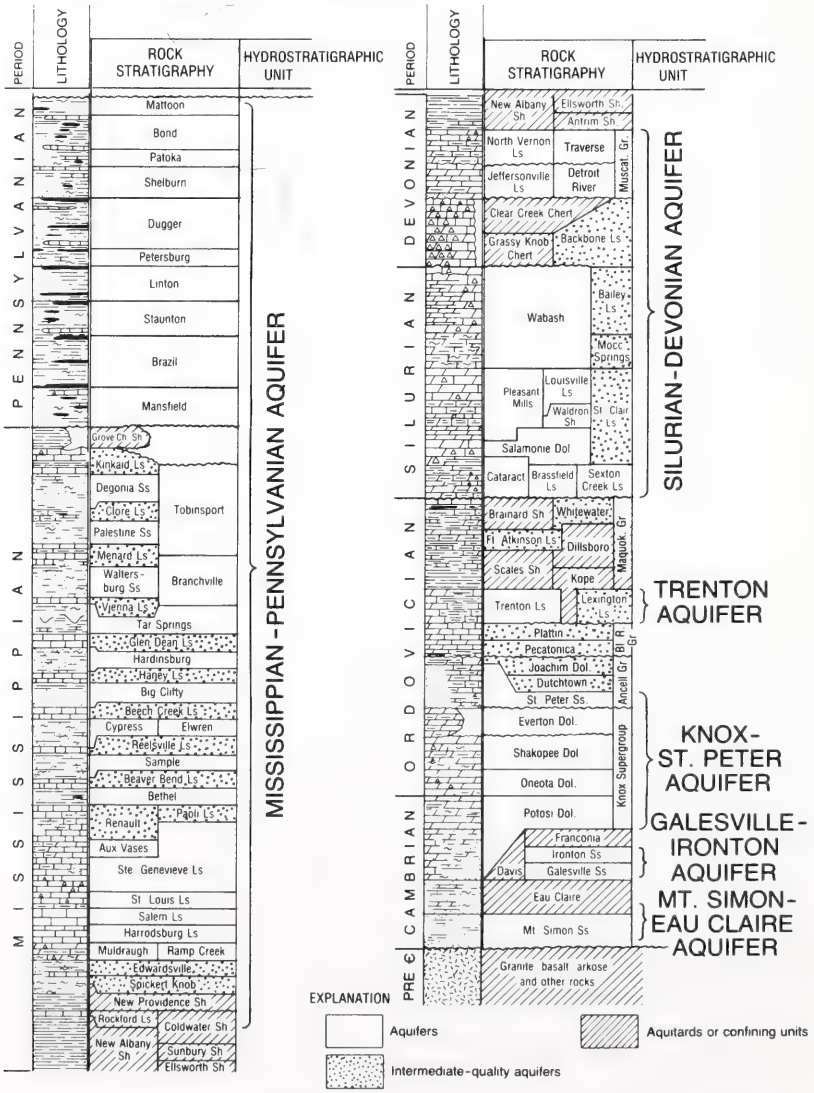

FIGURE 2. Generalized stratigraphic column for Indiana showing hydrostratigraphic units.

The factors that affect the geometry of this salinity transition surfaces are regional and localized structures, lateral and vertical permeability distribution, and recharge sources and flow gradients of aquifers. In a homogeneous porous medium with no external effects, the shape of the isosalinity surface will subparallel the topographic surface. The map of the 10,000 mg/1 TDS surface indicates significant influence from the above mentioned factors.

Structural influence on the salinity surface is dominated by the large regional low-amplitude Cincinnati and Kankakee Arches which divides the state into the Michigan Basin to the northeast and the Illinois Basin to the southwest (Figure 3). Because older sediments are structurally higher along the arch, the 10,000 mg/1 TDS surface occupies a lower hydrostratigraphic position along the axial trend. Smaller scale features such as faulting and fracture systems, may significantly affect the distribution of salinity in aquifers, most notably the Knox-St. Peter and Silurian-Devonian aquifers (Figure 4).

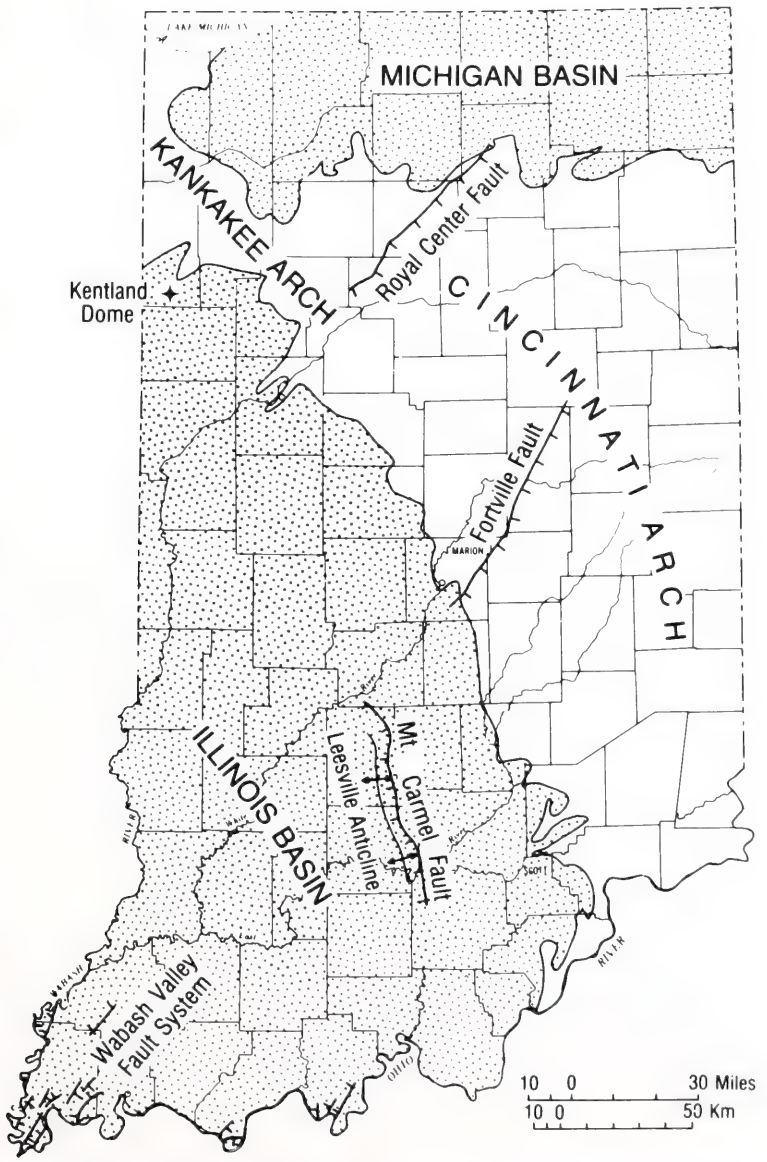

FIGURE 3. Map of Indiana showing major structural features. Basin margins are denoted by the erosinal limit of the Muscatatuck Group.

Vertical and horizontal heterogeneities in the rock column are a major control on the distribution of fluid salinities in the subsurface (Figure 2). Because the subsurface strata of Indiana are not a homogeneous porous medium but are a series of interbedded aquifers and aquitards with varying horizontal and vertical hydraulic transmissivities, the 10,000 mg/1 TDS surface "stairsteps" up the hydrostratigraphic column in an irregular manner (Figure 1). Horizontal variability in the permeability of various hydrostratigraphic units may be partially responsible for the different levels of fresh water infiltration in the Knox-St. Peter and Silurian-Devonian aquifers (Figure 4).

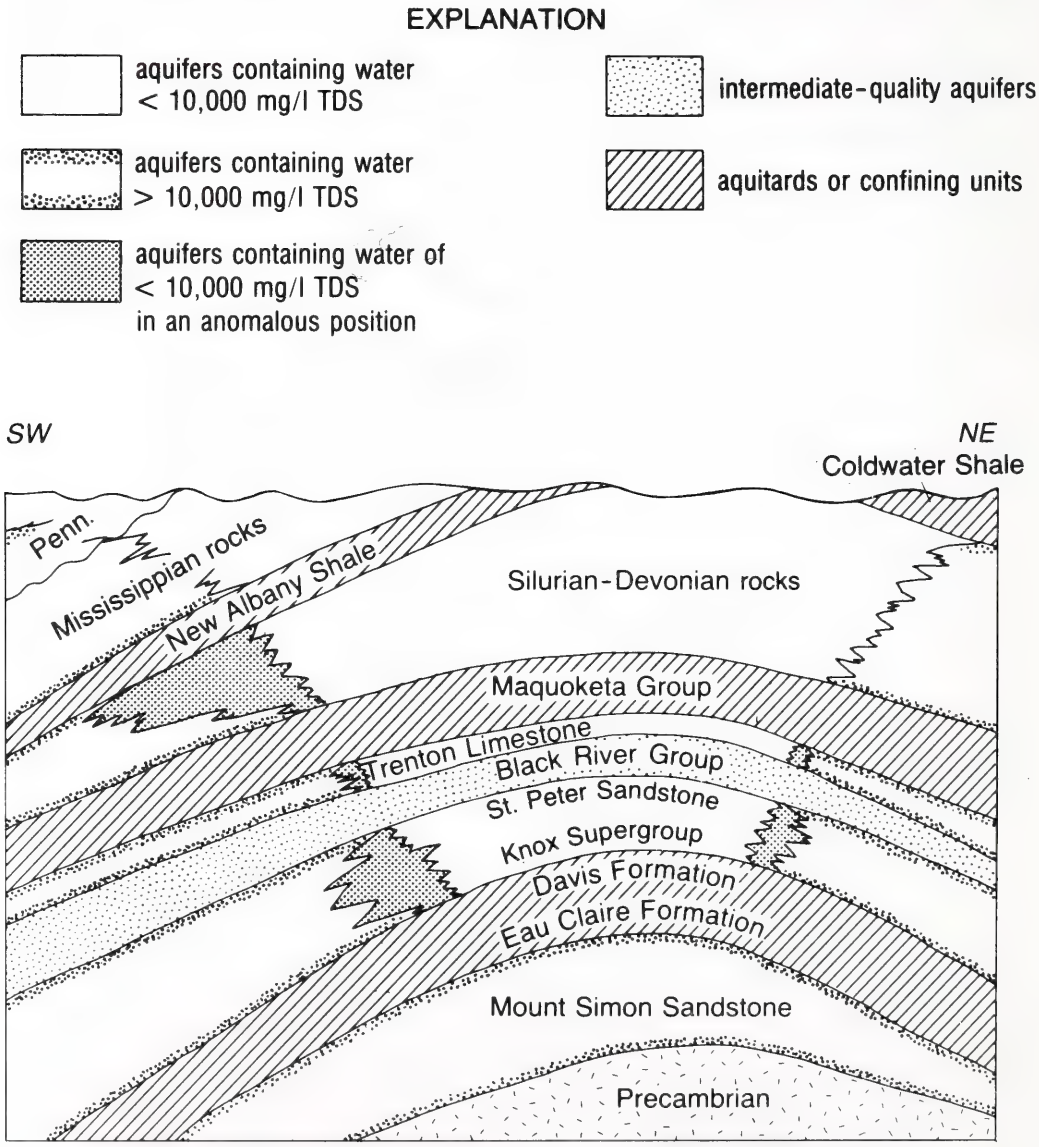

FIGURE 4. Diagrammatic southwest-northeast cross section showing the hydrostratigraphic position of the 10,000 mg/1 TDS surface. Waters of less than 10,000 mg/1 TDS plunge off of the axial trend of the Cincinnati Arch deep into the Illinois Basin.

The recharge and discharge areas of the aquifers also affect ground water flow and therefore the salinity distribution. Although most of the brackish and saline ground

water occurs in confined deep bedrock aquifers, the surface and near surface hydrologic framework also influences the salinity gradients. Large plumes of less than 10,000 mg/1 TDS ground water exist in the otherwise saline Knox-St. Peter and Silurian-Devonian aquifers. These plumes, which in some places underlie waters of greater than 10,000 mg/1 TDS (Figure 4), are potentially the result of increased recharge due to Quaternary glacial effects over large outcrop areas. Although some aspects of the direction and magnitude or regional flow in these bedrock aquifers have been established (6), the long-term effect on the salinity gradients has not been documented.

Summary

The final map (although generalized) shows the basic configuration of the 10,000 mg/1 TDS surface in Indiana. Local geologic circumstances will slightly modify this basic configuration as more data are acquired. There is a fair correlation between the regional structural patterns (Figure 3) and the position of the 10,000 mg/1 TDS within the hydrostratigraphic column (Figure 2). The structural influence on the position of the 10,000 mg/1 TDS surface in the hydrogeologic column is similar to the structural effect on the outcrop pattern of the stratigraphic units. Although the configuration basically conforms to regional structural fabric, significant deviations are evident. A notable deviation is the major southward-trending anomaly that shows fresher water occurring on the southwest side of the Cincinnati Arch deep in the hydrostratigraphic section. Anomalously fresh water is present in both the Knox-St. Peter and Silurian-Devonian aquifers within this area (Figure 1).

These plumes of fresh water may be the result of increased infiltration through fracture systems within the carbonate aquifers and (or) differential recharge that could have resulted from Quarternary glacial effects. With the addition of more tests in areas which have been historically non-productive of data from deep drill holes, an increased resolution of the geometry of the 10,000 mg/1 TDS surface will be possible. A clearer definition of the position of this surface will permit a more accurate assessment of the volume and distribution of Indiana's potentially potable ground water resource.

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George K. Greene: An Early Indiana Fossil Collector

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Introduction

George K. Greene (Figure 1) amassed one of the largest fossil collections ever accumulated from Indiana rocks. He spent most of his life in southern Indiana, first in Jeffersonville and then for many years in New Albany, Indiana. He is best known for the tremendous collection of silicified corals that he obtained from the Falls of the Ohio. At the time of his death his collection was estimated to have numbered over 400,000 specimens. He was initially introduced to fossils by Albrecht Koch, a German paleontologist who travelled extensively in the eastern United States during the middle 19th century. Greene worked for several years for the Indiana Geological Survey and later privately published an extensive series of taxonomic papers on corals and some other fossils from the Falls of the Ohio and other localities in southern Indiana. His collections posthumously went to the American Museum of Natural History in New York, where his type specimens still reside. The bulk of the collection was later transferred to the U.S. Natural History Museum in Washington, D.C.

Early Days

George K. Greene was born in 1835 in Columbus, Indiana. His father was George Graham Greene, a merchant of Hancock County, Kentucky. His mother was on a visit to Columbus when he was born, hence he was accidentally born a Hoosier, where he later lived. Greene received a public school education in Kentucky and he says that he also studied Latin and science with private teachers (1). At the age of 16 he met a well-known German paleontologist, Albrecht C. Koch, who was in Kentucky collecting geological specimens for a French nobleman who was to endow a College in his native land (1). Koch excited Greene's interest in rocks and fossils and the boy left home to travel with Koch and to "become a geologist". Greene

FIGURE 1. *Photograph of George K. Greene, taken beside his fossil shop in New Albany, Indiana. Date unknown. Copied from photograph in Miss Emma Carleton scrapbook no. 1, page 11 in the New Albany-Floyd County Public Library, 180 West Spring Street, New Albany IN 47150, courtesy of Ruth Ann Kramer.*

says that he was with Koch for several years, but the exact duration of this association, where they travelled and what they did, is not recorded.

Koch worked primarily out of St. Louis, where he had established a museum in 1836 (2). He was responsible for excavating and naming an American mastodon from Missouri in 1839. He called the specimen *Missourium* or the Missouri Leviathan. He assembled parts of two skeletons into a single super-mastodon and had it on exhibit. He later shipped the fossils to Europe and sold the specimen to the British Museum, where the anatomist Richard Owen correctly mounted the specimen.

Later, in 1845, Koch excavated an enormous Eocene age archaic whale said to be 119 feet long. Most specimens of these whales are 70 feet or less in length. He called this "sea serpent" *Hydrarchos* even though the whale had earlier been named *Basilosaurus* or *Zeuglodon*, names that are in use generally today. One of his whale skeletons was later sold to the Royal Museum of Berlin.

Presumably Greene returned to Kentucky and remained there until middle age. He married, his wife's name was Elizabeth, and he had at least two sons born in Kentucky, Newton A., later Mayor of New Albany, and William (3). In 1870, at age 35, Greene moved to Jeffersonville, Indiana so that he could be situated closer to the famous fossil beds at the Falls of the Ohio. Eight years later, in 1878, he moved permanently to New Albany. The Devonian limestones at the Falls, which form rapids across the Ohio River between Clarksville, Indiana and Louisville, are

famous for the size, abundance and variety of fossils, especially corals and sponges (stromatoporoids) found in them. The beds were noted by early explorers of the Ohio River and species of fossils were first described from the locality in 1820 by Rafinesque and Clifford (4).

A Collector

Upon moving to Indiana Greene began putting together the large collection of fossils and antiquities that was his principal claim to fame.

Many of the fossils from the Falls of the Ohio occur in limestone and have been replaced by silica. Thus, they weather out of the rock as the limestone dissolves and the more chemically resistant silica remains behind. The fossils commonly accumulate in potholes worn in the limestone in the bed of the river and these potholes, some several feet in diameter, were favorite places for early fossil collectors to obtain fossils. Many of Greene's specimens were undoubtedly obtained in this way. It is quite clear that Greene knew how to help Nature along, as he mentions treating pieces of limestone that contained fossils with muriatic acid (commercial hydrochloric acid). The acid dissolved away the lime rock, thus releasing the fossil (1). Greene must have been one of the earliest paleontologists to employ this technique for studying fossils.

Although corals from the Falls of the Ohio were clearly Greene's speciality, his advertising card states that he also dealt in archaeological specimens, scientific books, coins, medals, badges, U.S. and foreign postage, revenue stamps, Confederate and broker's bank bills, as well as old arms and relics of the late [Civil] war (5). His shop was located at 25 West Market Street in New Albany. In 1911, when he gave an extensive interview with the Louisville Journal newspaper, he lived in two small rooms in the back of this shop.

In this interview (1), at age 76, Greene says that he had sold specimens to the American Museum of Natural History, the British Museum of Natural History, the Museum of Comparative Zoology at Harvard, the Boston Society of Natural History, and to the Massachusetts Institute of Technology. He also states that he had sold a 150 pound coral head to the Borden Institute at Borden (then New Providence) Indiana for $50 and also sold a fine crinoid to Professor W. W. Borden, founder of that Institute, for $100. He had found tusks and other fragments of a mammoth at the Falls. In this long interview Greene also expresses some peculiar ideas on the origin of the ice ages, on extinction of large mammals, and on religion.

Shortly after moving to New Albany, Greene spent some time during 1878 in Bloomington, Indiana, where he was employed to catalog and arrange the David Dale and Richard Owen collection of fossils and other geological specimens at Indiana University. These collections were later, in 1883, almost completely destroyed by fire. In the next year, 1879, Greene went to work for the State of Indiana. He was appointed as an Assistant on special duty in the newly formed Department of Statistics and Geology. He undertook the first and only geological survey of the geology of Monroe County. The report was published in 1881 (6).

From 1881 through 1883 Greene was employed in Indianapolis where he continued to work for the State geological survey, renamed the Department of Geology and Natural History. He was responsible for arranging the State geological and paleontological collections in the State Museum. His title was changed from Assistant on special duty to Museum Assistant. He oversaw expansion of the collections from about 40,000 specimens in 1881 to an estimated 100,000 specimens by 1883, with an estimated value of over $100,000 (7,8). During this time he was paid about $65 a month in salary as well as expenses for travel to and from New Albany. He did fossil collecting in Shelby, Cass, Carroll and White counties as well as at Waldron,

Hartsville, and Logansport. He ceased work for the State in January, 1883, probably because of budgetary restrictions. In 1885 Greene became one of the founding members of the Indiana Academy of Science (9).

Contribution to Indiana Paleontology

Amateur paleontologists such as Greene have a great desire to see their collections receive notice. Having gone to a great deal of time and expense to collect the fossils, they are especially anxious that any new forms, genera or species, be named and published. They can seek out a professional who is willing to write up their new taxa. Alternatively the collector may decide to take matters into his own hands and publish on the specimens himself. This was the path chosen by Greene and the motivation behind his *Contribution to Indiana Paleontology*, which is basically a description and illustration of parts of Greene's very large fossil collection (10).

Greene began publication of the *Contribution* with part I on Feb. 28, 1898. In the preface he states that he will publish in parts that include three plates each, that he has enough fossils for 60 plates, that each part will be for sale for 25 cents and that 500 copies will be printed. True to his word, six years later on Sept. 20, 1904, he issued part XX, resulting in a total of 60 plates of fossils. After a two year interval he published volume 2 of the *Contribution* in three parts between July and November, 1906. Each of these parts also had three plates (10).

Greene's collection was heavily weighted toward fossil corals, especially those of Devonian age from the Falls of the Ohio and other areas near New Albany where he lived. Thus, as one might expect, the *Contribution* is also preponderately concerned with Devonian corals that Greene believed were new species, and in some cases, new genera. Of as total of 356 species discussed in the *Contribution*, 181, or over one half, are of corals.

The first six parts of the *Contribution* are devoted exclusively to corals. A total of 86 species are described, all but one of which are thought to be new. Each of the subsequent 13 parts invariably begin with some coral descriptions, ranging from two to nine in number, but also including a variety of other fossils. All descriptions other than corals, which Greene did himself, were prepared by R. R. Rowley of Louisiana, Missouri. Finally, in part XX Greene reverted to sole description of 11 more new species of corals. In volume 2, the first two parts include other fossils but the third and final part contains 10 descriptions of new coral species.

Of the 181 species of corals described in the *Contribution* only one was not described by Greene. In part VIII one coral is described by Rowley and named *greenei* for George Greene. Apparently Greene wanted one species named after himself and could not, with proper taxonomic etiquette, do so himself, so he had Rowley do it for him.

Of the 181 species of corals described only 15 were assigned to species previously described by earlier workers. Greene essentially ignored all previous work, which was extensive, on Falls of the Ohio corals. Corals had been described from this famous fossil locality as early as 1820 when Rafinesque and Clifford published on some fossil corals (11). Later paleontologists have emphatically disagreed with Greene concerning the appropriateness of his new species. In a monographic revision of the Falls of the Ohio corals by Stumm in 1964, all but 15 of Greene's 166 new species of corals are placed in synonymy with previously described species (12). Thus, a major part of the *Contribution* is viewed today as a futile exercise in species making.

Rowley was much more conservative in his species making than was Greene. Of 95 crinoid species described, 49 were assigned to earlier named species and 28 of 42 species of blastoids were not new. Today Rowley's work in the *Contribution* has considerably more scientific value than does Greene's.

Greene used a considerable number of illustrators to prepare the plates for the *Contribution*. Even by standards of the time most of the figures were not of very high quality and some are so generalized as to be virtually useless for purposes of identification. John Bridgham of Providence, Rhode Island was the sole artist for the first four parts. He later provided two plates for part V, two for part VII, and one for part VIII in 1901. All of these plates were of corals. Albert Albers of Cincinnati, Ohio sporadically drew a series of plates beginning with part V and ending with part XII, for a total of eight plates.

Beginning with part VII and Rowley's contributions, Rowley also began preparing plates. Those that he did prepare are of especially poor quality and are without exception of fossils that he also described. Beginning with part VIII Fred B. Stichter began to prepare plates for Greene. His drawings were generally of fossils other than corals. He prepared as total of 12 plates for parts VIII through XVII. In latter parts of volume 1 and in volume 2 plates were drawn by P. W. Alles and J. M. Paine. Nothing further is known about these illustrators.

With part XIII Greene began to use photographic illustrations of fossil corals. In each case all specimens comprising figures for a plate were carefully arranged and a single photograph was taken of the entire assemblage. Those photographs are invariably the first of three plates in each part where they appear and are always of fossil corals. The photographic images are of considerably higher quality than the various illustrators' sketches. No credits are given for the photographic plates and they may have been taken by a local New Albany photographer or by Greene himself.

Among the many new species named by Greene quite a few were named for other collectors or paleontologists and these give us some insight into other early fossil collectors in the New Albany area. Species are named for R. R. Rowley, Albert Albers, and John Bridgham, all of whom collaborated with Greene and who were discussed earlier. Local collectors known to Greene who provided specimens that he described and that became part of his collection included W. W. Borden, Thomas J. Lamaster of Speed, IN; Dr. John Lemon of New Albany; John Hammell of Madison, IN; John Sellers and William N. Williams, collectors; Dr. Otto Oppelt, New Albany; George Weber of Louisville, Charles Very of New Albany and Henry Grossback of Sellersburg.

A second group consisted of amateur and professional paleontologists at a distance who may have traded or bought and sold specimens with Greene. These included W. F. E. Gurley of Danville, IL, Rev. H. Currie of Thedford, Ontario (a prime locality for Devonian corals); Dr. Marshman E. Wadsworth, former President of Michigan Mining School; Prof. A. E. Seaman, Professor of Natural History at the same institution; and Prof. Will. H. Sherzer, Prof. of Natural Sciences at the State Normal School, Ypsilanti, Michigan. The emphasis on Michigan folks, none of whom were well-known in paleontological research of the time, may have been due to the richness of Devonian fossil localities in that state, especially corals.

A third category consists of very well known paleontologists with whom Greene probably had little personal contact. These include Amadeus Grabau of Rensselaer Polytechnic Institute; Alexander Agassiz, Harvard University; Wm. H. Niles of M.I.T.; Charles E. Beecher of Yale; R. T. Jackson of Harvard; Frank Springer of New Mexico and Stuart Weller of the University of Chicago. Finally, one species is named *hobbsi* with no indication of who Hobbs might be.

Disposition of the Greene Collection

George K. Greene died in New Albany in 1917 at age 82. In September of that year his son Newton contacted the American Museum of Natural History concerning

sale of his father's collection (13). Newton Greene valued his father's collection at $7500 and offered to sell it for $2500 down (cash) and $1500 per year for three years at 6% interest. He would take a substantial reduction if paid in one lump sum. He also indicated that at one time the Field Museum of Natural History in Chicago had offered his father $10,000 for the collection but that negotiations had failed.

The American Museum sent Dr. C. A. Reeds to New Albany to inspect the collection and to arrange for its purchase. He wrote that the collection consisted of 1500 boxes, 100 cabinet drawers, 3 show cases, 5 bins, 65 cigar boxes, 1 trunk of Indian relics, 3 cabinets and 20 bushels of fine specimens. These were very dusty and scattered through eight rooms of a "fire trap haunted house". The museum bought the collection for $5500 and Reed packed the specimens in 5 sugar barrels and 134 wooden boxes, all together weighing 23,000 pounds.

At the same time Reed purchased another Falls of the Ohio coral collection from Wm. J. McConathy of Louisville consisting of over 7000 specimens for $2300.

The Greene collection was accessioned (AMNH No. 490) but was stored and never curated except for the type specimens. In 1960 the entire collection except for the types was donated to the U.S. National Museum, where it is housed in 21 3-foot range cases (14).

In summary, George K. Greene amassed one of the largest fossil collections ever assembled by a Hoosier. He published a series of papers describing new species, especially Devonian age corals from the Falls of the Ohio, from his collections. Virtually all of these species have since been declared to be junior synonyms of earlier described species. He was an early user of hydrochloric acid to release silicified fossils from limestone matrix and early used photographic plates to illustrate fossils.

Acknowledgments

I am especially indebted to Ruth Ann Kramer of the New Albany-Floyd County Public Library for much valuable assistance. I also had help from Ernest Greene, Walter Nance, William A. Oliver, Ernst A. Stadler, Roger Batten and Sydney Horenstein.

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Clark Wissler, Artifact Collector

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Clark Wissler, a birthright Hoosier, was born in 1870 near Hagerstown. He attended Indiana University from 1893 to 1897 but obtained his anthropological training on the East Coast. He was one of the half dozen most prominent anthropologists of the American Historical School and viewed as the anthropological authority of the American Indian during the 1920's and '30's. He was introduced to archaeology during his Indiana upbringing.

Citing Barton, the Freeds note (1, p. 803) that Wissler let it be publically known that he was a childhood collector of Indian artifacts:

> His [Wissler's] birthplace was a rural village of six houses surrounded by farmland. And it was the owner of one of these farms, an industrious collector of Indian artifacts, who inspired him to take up the study of aboriginal life. . . . After school the boy [Wissler] would tramp about the fields unearthing arrow heads and other more complicated specimens. . . . His farm-tutor had evidently read up on these artifacts in the anthropological literature of the day, and was so conversant with their history and uses that his pupil *had little to unlearn in later life* (italics and brackets - BKS) (2, p. 48).

This raises the question of what Wissler's view of non-professional archaeological surface collecting was after he became an established professional anthropologist. This is no secret. Wissler states:

> *Every boy* can explore the country near his home. Inquiry among the farmers will often aid him in locating probable village sites, since those men may know where relics are most abundant. The best time to go over the ground is in the spring, after the winter rains have cleared the surface and before the grass is high. Take *any* field, and walk along one side, sweeping the surface continually with your eyes; examining every suspicious-looking object. . . . Another good place to seek relics is along the banks of streams. The spring rains usually bring freshets that cut into banks and expose new surfaces. . . . They appear as streaks on the face of the fresh-cut bank; they usually show ashes and charcoal. . . . you may note. . . . cave-like places under ledges of rock. . . . The best thing you can do is *scrape away* the leaves and other refuse. . . . and look carefully over the ground (italics - BKS) (3), unsigned; but authorship credited by Murdock (4, p. 297).

Indeed the Departmental Editor of *The Youth's Companion* was apparently unaware of this facet of Wissler's background, or considered it as useful qualification for the article he was soliciting Wissler to write, but chose not to mention it (5). In 1941 Wissler submitted a manuscript, co-authored with Junius Bird, entitled "How to be an Archaeologist" to McBride & Co. for publication (6). Wissler's nostalgia of his boyhood interests is apparent in a brief sketch incorporated in an insightful methods article (7) from the New York *Times*, about a 13 year old boy reporting the existence of an undisturbed cave he discovered to the archaeologist at the Trailside Museum, Bear Mountain Ridge, New York.

Apparently Wissler supported and even encouraged surface collecting by non-professionals. This is quite curious since Wissler's view of archaeology was far from

antiquarian in precept. His knowledge of archaeological method was quite sophisticated (8) and he viewed the role of archaeology in anthropology as important and significant:

> The great triumph in culture research will come when a worldwide chronology is attained, a time-sequence scheme that is workable and verifiable. True, there have been attempts, rough outlines of the evolution of culture have been sketched, but these are for the most part guesses; they can never serve as foundation for genetic interpretations. Yet the problems involved are, for the most part, just as objective as those of geology, and equally immune to all onslaughts except by the most refined technique. If, as has been said many times, science is the experimental and inductive determination of the hidden relations between things, then the true archaeologists deserves to sit among the great; for by infinite patience, amid a bewildering maze of details, he proceeds step by step to the discovery of time-relations in the debris. . . . since eventually one such increment after the other will fit into one unifying sequence" (9).

Wissler viewed archaeology as, perhaps, THE method of reconstructing culture history, though the process is slow and almost painful. It is interesting that despite his high regard for archaeological method and his unchanged interest and research in American Indian material culture Wissler never conducted any organized professional archaeological field work. He was versed in archaeological method, theory and goals, and often wrote general accounts of other archaeologists' research, especially his colleague Nels C. Nelson, to whom he apparently had high regard. In fact it was Nelson's work that Wissler used to try to demonstrate his concept of "natural diffusion," diffusion that spread at a uniform rate (10, Fig. 9-3, p. 338). Wissler published 26 works dealing with data primarily of an archaeological nature. He was also active in the administration of archaeology, for example Committee on State Archaeological Surveys (NRC), Committee on Basic Needs in American Archaeology (NRC) and the American Archaeological Survey of Asia Minor.

It is clear Wissler's archaeological method and theory were sound, but, one fears, his views on who should be the practitioners may be suspect.

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Zoology at Indiana University: The Torrey Years

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ABSTRACT

Theodore Willett Torrey died August 31, 1986, after a long career in teaching and administration at Indiana University, Bloomington. His research, his innovations in teaching, his work on university committees, and his distinguished administration did much to raise the status of zoology at Indiana University locally and in the national and international community. Torrey's contributions are summarized along with a chronology of the major changes during his administration of the Zoology Department.

From July 1, 1948, when he was appointed Chairman of the Zoology Department until his retirement from administration July 1, 1966, Theodore Willett Torrey was untiring in his support and management of the Department and his wife, Marcella Loge Torrey, was a gracious hostess at many social affairs involving their friends and associates.

Torrey died August 31, 1986, having had a career distinguished for its devotion to Indiana University and the field of zoology. Elsewhere (Young, 1983), I have paid tribute to Ted as an administrator. Within his field of activity he had no superiors and few if any equals. He kept a varied group of teachers and research workers headed in essentially the same direction despite such varied personalities that disagreements were often unavoidable. He could have written a book on the care and nurture of intellectual tigers while at the same time he found time to keep us, the lesser scientists who never attained international fame, from feeling neglected or underprivileged.

Torrey came to Indiana University in 1932 at the invitation of Dean and Chairman of Zoology Fernandus Payne, who recognized that future progress in zoology lay largely in the direction of the experimental aspects. Torrey completed his doctoral work at Harvard University earlier in 1932 and came to I.U. in the fall. He soon introduced the concepts and methodologies of experimental morphology into the biology curriculum. He took up the development and function of the vertebrate kidney, including that of humans, and continued to do meticulous and distinguished research in that field for many years.

Torrey's research interests also influenced his teaching. He soon developed a dynamic approach to the study of the origins of vertebrate organs which culminating following World War II in the union of embryology and comparative anatomy into developmental anatomy. This innovative approach was presented in his textbook, *Morphogenesis of the Vertebrates* first published in 1949. The union of anatomy and embryology is now widely followed in many universities and colleges, and has proven valuable to the teaching of these often complex subjects. The approach also laid the groundwork for many more sophisticated studies of vertebrate development by other workers.

Torrey was a meticulous teacher who set high standards for his many students.

He pushed them hard, and in return received respect if not love. "Terrible Ted" was one of the more refined nicknames he received. W.R. Breneman (1972) asserts that a typical comment from his students in later years was that T^2 (=T Square) taught us the most valuable course we had as an undergraduate. Others, it is only fair to say, were not so complimentary.

In addition to his services to the University through his administration, Torrey was also influential in his chairmanship of the All-University Curriculum Committee. This committee was instrumental in making many University wide changes in course and degree requirements. It reported at its own discretion to the President, the Dean of Faculties, the Faculty Council, or the schools and departments and helped to guide the expansion of Indiana University during the rapid growth between 1953 and 1965. One notable accomplishment of this committee during Torrey's chairmanship was the acceptance of a basic curriculum by all divisions of the University. The committee also ruled on the acceptibility of specific courses in satisfying the requirements of the basic curriculum. This curriculum was approved by the Faculty Council and the administration. It has never been repealed although individual colleges and schools have built upon it.

Again W.R. Breneman (1972) comments that Ted's "patience, foresightedness, and fairness during the proceedings (of this committee) made the innovations recommended. . . . more acceptable to those who dissented." Later Torrey was actively involved with the committee which led to the transformation of the Junior Division into the University Division.

Torrey's research and teaching was a great contribution to the field of Zoology, but his most outstanding contribution was through his 18 years of devoted administration of the Zoology Department. His cooperation with other departments in the biological sciences led to the introduction of cross-departmental courses which have made major contributions to the broadening of the training of university students.

Ted's administrative technique was firm but quiet. I do not believe in the 18 years I was associated with the Zoology Department during his administration we ever took a departmental vote on any matter. Ted quietly interviewed each of us and reached a conclusion on which he acted. Neither do I remember any really important matter on which he did not manage to reach a conclusion satisfactory to most.

On the occasion of Ted's retirement W.R. Breneman wrote (1972): "My relationship with him as a colleague and friend has been an enriching experience. All of us in biology at Indiana University, past and present owe an inestimable obligation to him for his effectiveness in advancing the biological sciences and for his leadership in undergraduate education at (Indiana) University. His has been a good life, a rich and rewarding life, and we wish him well in the years to come." All of us who were in the Department of Zoology during Ted's tenure as chairman echo this endorsement.

In 1948 when Torrey assumed the chairmanship of Zoology, the department contained the following faculty members: W.R. Breneman (Endocrinology); P. Sears Crowell (Invertebrate Zoology); S.D. Gerking (Limnology); A.C. Kinsey (Sex Research); L.A. Krumholz (Limnology); H.J. Muller (*Drosophila* Genetics); Fernandus Payne (Cytology); W.E. Ricker (Liminology); Tracy M. Sonneborn (Protozoan Genetics) and W.J. vanWagtendonk (Biochemistry). Lamont C. Cole (Ecology) had been a member of the department for the preceding two years. Brief biographies and summaries of the research of the preceding are recorded in Young (1983). The writer, Frank N. Young (Ecology, entomology, and parasitology), joined the Zoology faculty in the fall of 1949.

During the Torrey years various members of the faculty left and new ones were appointed. The following is roughly the sequence.

Aquatic Biology

David G. Frey was appointed in June, 1950 to replace W.E. Ricker who returned to Canada. Frey besides teaching courses in the department and carrying on his own research on Crustacea directed the Indiana Lake and Stream Survey. Its ups and downs were accompanied by several changes in personnel. Robert Wetzel (Ph.D. California) was director from 1962-1965. Joseph Nelson (Ph.D. British Columbia) was director from 1956-1968, and F. Douglas Martin (Ph.D. Texas) from 1968-1971. In the meantime Shelby D. Gerking departed to become chairman of zoology at Arizona State University.

Experimental Embryology

James E. Ebert joined the zoology faculty in 1951, and departed for the Carnegie Institution in Washington, D.C. in 1956. He was replaced by Robert W. Briggs in 1956, who continued to teach and do research at Indiana until his death. This was an important period in the development of Zoology at Indiana in regard to experimental embryology (See Young, 1983).

Genetics

The retirement of H.J. Muller left a gap in the departments curriculum in genetics. This was filled in part by George Hudock (Ph.D. Harvard) who joined the department in 1965. Dr. Muller's research and teaching had been mainly in the field of *Drosophila* genetics, but Hudock expanded the teaching to bacterial, algal, and protozoan genetics as well. His chief interest is in the genetics of *Chlamydomonas* and *Euglena* and the control of gene action. He developed a strain of *Chlamydomonas* which lacks chlorophyll and was thus a heterotroph. Is he a zoologist working on plants or a botanist working on animals? Recently, his attention has turned to a genetic dissection of phototaxis and gametogenesis in *Chlamydomonas reinhardi*. Mutants, most of which are conditional, have been isolated which affect both of these phenotypes. Among his more practical accomplishments, he isolated a strain of bacteria for the U.S. Navy to biodegrade TNT. He claims his intent was peaceful. He also isolated a strain of bacteria from the floor of the Atwater Garage on the I.U. campus which biodegrades motor oil. The latter was destroyed since it was a pathogen. His current research involves the question of why living material became multicellular in the first place since there are advantages and disadvantages. The Volvocales are, for example, essentially multicellular Chlamydomonads. He has selected mutants which are unicellular at 25 °C but which form clumps which are sometimes regular at 35 °C.

Other workers in Microbiology and Botany also contributed to the development of genetics at Indiana University.

Endocrinology

Frank J. Zeller received the Ph.D. from I.U. in 1957 and joined the faculty that year. His research is mainly on the endocrinology of reproduction. He has been honored as an outstanding teacher and was Acting Chairman of Zoology in 1975-76.

James P. Holland received the Ph.D. from I.U. in 1961 and after postdoctoral work at the University of Wisconsin and several years of teaching and research at Howard University returned to I.U. in 1967. He has contributed greatly to I.U. through his service on committees and as an assistant dean of the graduate school. His research interests also are primarily in the endocrinology of reproduction. He has also been recognized as a distinguished teacher.

The Kinsey Institute for the Study of Sex, Gender and Reproduction

The death of A.C. Kinsey in 1956 required some adjustments of the research unit which he had established at Indiana University. The salary of the director and some of the other financing was still routed through the Zoology Department. Thus Torrey was required to assist with the organization after Kinsey's death. In 1966, upon his retirement, Torrey wrote as follows:

"Although the Institute for Sex Research (see expanded title above) has been a largely autonomous organization, by reason of two simple facts its history has been linked to that of the Department of Zoology. First, Dr. Alfred C. Kinsey, its founder and first director was Professor of Zoology; therefore many aspects of the Institute's early development routinely came under the purview of the chairman of the Department. Second until very recently a small segment of the Institute's finances (in addition to Dr. Kinsey's salary) resided in the Departmental budget and to that extent the Institute was a departmental enterprise. . . ."

"The work of the Institute began on a relatively modest scale with financial support from the Medical Division of the Rockefeller Foundation and the National Research Council's Committee for Research on Problems of Sex. Its working quarters consisted of Dr. Kinsey's original office-laboratory plus two small adjacent rooms in old Biology Hall. The Institute's first major publication, 'Sexual Behavior in the Human Male' (Kinsey, Pomeroy, and Martin), in 1948 brought immediate changes. A hot glare of publicity descended upon Kinsey and his co-workers and the University itself and with it, controversy and debate. In show-business parlance, Kinsey became a 'hot property' for the lecture circuit. Sizeable royalties from the book nurtured an enlargement of the Institute's activities with the accompaniment of additional personnel, increased library acquisitions, and diversification into art and anthropological relics. All this called for more working space, so early in 1949 the University administration agreed to relocate certain occupants of Wylie Hall and completely remodel the bulk of the basement of that building for the Institute. The job was completed during the following year and the Institute shifted to Wylie in April, 1950, there to remain until the opening of Jordan Hall in 1955.

"Work within the Institute proceeded apace and in 1953 the second major book, 'Sexual Behavior in the Human Female' (Kinsey, Pomeroy, Martin, and Gebhard), was published. Its appearance elicited, happily, less titillation from the general public; more importantly, to professional readers the book went far toward meeting the criticisms of statistical analyses that had been leveled against the earlier one. Volume two, however, was only a way station along a road of additional projects which had been mapped, and almost without pause Dr. Kinsey moved ahead with unabated energy.

"But unremitting 18-hour working days and an exhausting schedule of travel and lectures took their toll. Kinsey's rugged physique began to crack under the strain and in June, 1956 he suffered a heart attack. I recall so vividly a conversation I had with him early in August. I urged him to think of his responsibility to himself, his family, and to his colleagues and to enter into rest and relaxation for whatever period required to restore his health. As he walked toward my office door he turned and said: 'I'll think about it, but if I can't work, I'd rather die'. That was to be the last time I would talk with him; he died on August 25, 1956. I can think of no better final words about him than some of those I spoke on the occasion of his funeral.

> "It is not my intention to trace the history of the present Institute, much less to outline the nature and scope of its program so well known to you all. I wish, rather, to dwell for a moment on the qualities of this man, Alfred Kinsey, which made it all possible.

"There was boundless energy for the labors of every order of magnitude. There was consummate skill in gaining the confidence and respect of persons in all walks of life. There was integrity and devotion to objective science that commanded the support and cooperation of fellow professionals in all areas of human endeavor. There was courage in the face of prejudice and misunderstanding. There was loyalty, reciprocated in kind, to the members of his immediate staff. There was breadth of vision requisite to the building of the broad foundation upon which the Institute rests. Withal, there has derived a monumental record on human behavior which now and in the future will go far toward aiding man in understanding himself.

"Dr. Kinsey's death posed the immediate problem of the future of the Institute. The question was not whether it should continue; rather, how it should be administered. An initial suggestion was for the creation of a co-directorship involving Paul G. Gebhard (Anthropology, Ph.D. Harvard) and Wardell Pomeroy (Psychology, Ph.D., Columbia) both long associated with the Institute. It was shortly decided, however, that leadership should be vested in Gebhard as Executive Director, with Pomeroy to have the subsidiary title of Director of Field Research. In order to maintain the connection with the Department of Zoology, I myself was added to the Board of Trustees. Further tie with the Department was created by an administrative decision to place the counterpart of Kinsey's salary in the Zoology Budget for use, following my review and approval, in bringing in outside research consultants from time to time.

"Since the pioneering days of data acquisition were largely over even before Kinsey's death, the character of the work of the Institute had already begun to change; his death only hastened the process. Acquisition of data through interviews and other devices was not to stop, nor has it ceased to this very day, but emphasis was to be placed more on the evaluation of the data already in hand with respect to special problems dealt with only in passing in the first two major publications.

"There were thus published in 1958 a modest-sized volume on 'Pregnancy, Birth, and Abortion' (Gebhard, Pomeroy, Martin, and Christenson) and a very sizable one in 1965 on 'Sex Offenders' (Gebhard, Gagnon, Pomeroy, and Christenson). (Incidentally, a change of publishers may be noted. The original two volumes on male and female behavior were brought out by W. B. Saunders Co.; the latter by Harper and Row.) A change of complexion was also furthered by the recommendations of numerous external consultants, a change in three respects carrying down to this very day; (1) financial structure; (2) research projects; (3) staff and administration.

"1. Whereas, after its original subsidies had run out, the Institute subsisted largely on its own resources derived from book royalties (not counting the University's sizeable investment in physical plant and equipment, plus some lesser amounts already noted), it was clear by 1963 that new sources of revenue must be sought to replace the rapidly declining royalties. The solution came in a sizeable grant (1965) from the Mental Health Division of the National Institute of Health. . . . Lesser amounts subsequently came from various private foundations, sales of magazine articles and paper-back book rights, and gifts. The Indiana University Foundation has also provided some support and, as we shall see in another connection, shifts in certain salaries to the University salary structure have been made or are projected.

"2. An early high-priority project, following the acquisition of funds from the National Institute for Health, pertained to data-retrieval. In fact, the project was a recommended requisite for the granting of NIH funds. The over 18,000 case histories in the Institute's files were originally transcribed by a position code and abbreviated writing that for reasons of security could be known by only three or four persons. Quite aside from the inefficiency of the system, it was essential that the data be

transferred to punch cards and/or computer tapes in order (1) to make the data more widely available to Institute members and (b) to make the data available to outside scholars while still preserving the anonymity of the individual cases. A second project consisted of determining which of the persons whose cases are a part of the record had died since they had been interviewed and to determine what, if any, relationship existed between sexual patterns and cause of death. More recent studies are those on the social mores of college youth, sex education, and homosexuality.

"3. A consideration of staff and administration necessarily brings some overlap with budget and research. It is necessary, too, to confine attention to the principal personnel and related developments to the exclusion of the host of supporting workers—secretaries, code clerks, librarians, statisticians, photographers—as well as workers in other locales who have collaborated with or consulted with Institute workers. We have already recorded the principals identified with the first published volume, Kinsey, Pomeroy, and Martin. The last-named, Martin, was still an undergraduate when he became associated with Dr. Kinsey. He left the Institute in 1960 to undertake graduate work at John Hopkins. Following a year's leave of absence, Pomeroy left the Institute in November, 1964 to set up a private counseling service in New York. From 1950 until her recent retirement, Mrs. Cornelia V. Chistenson played a prominent role as Research Associate and her name is attached to the book on abortion and sex offenders. Although no longer an active worker, Mrs. Christenson remains on the Board of Trustees. John H. Gagnon joined the Institute as a Research Associate in 1959, assuming also a part-time appointment in the University's Department of Sociology. In addition to his collaboration on the book on sex offenders, Gagnon has been identified. . . . with the data retrieval project and to a lesser extent with the college youth study. He left the Institute to join the Sociology Department of the University of New York at Stoney Brook. William Simon became Research Associate in the Institute and Assistant Professor of Sociology in 1965 and has participated in three projects, college youth, sex education, and data retrieval. He, too, has left to join the Institute for Juvenile Research in Chicago, Dr. Alan P. Bell, with a broad background in theology and counseling psychology became associated with the Institute in 1966 and is currently developing a project on sexual development during childhood and adolescence, as well as serving as field director for the project on sexual deviance. Albert Klassen joined the Institute in 1966 for a two year stint as Research Sociologist, to be concerned primarily with data analysis. The newest addition to the staff will be Dr. Martin Weinberg, formerly on the faculty at Rutgers University, who will join the Institute in September. He will also be an Associate Professor in the Department of Sociology.

"One of the recommendations made as a contingency of NIH support was that the decidedly insular character of the Institute should be broken down through the creation of an Advisory Board composed of persons external to it. It was felt, and rightly so, that policy decisions which had always been made internally should have the benefit of detached, objective views of individuals unconnected with the Institute. After all, Kinsey himself had made most of the original decisions and except for me, all the trustees have always been workers in or closely identified with the Institute, an arrangement conducive to myopia. Such a Board, consisting of nine members derived from such diverse fields as psychiatry, education, sociology, folklore, and art was created in 1965 and is now regularly consulted.

"Any organization whose life depends upon the availability of funds from outside sources, federal agencies or otherwise, leads a precarious existence. At the very least, there is the recurring necessity to 'sweat out' applications for renewal of grants and much time and energy must be diverted from basic obligations to the never-ending search for money. Accordingly, these last two or three years have seen a search

for some means of establishing financial continuity and stability, a search that promises to recast the Institute's relation to the University. As a first step, part or all of the salaries of certain of the principal investigators have been assumed by the University; more specifically, their salaries lie in the budgets of the departments, e.g., anthropology, sociology, in which they hold joint appointments. The next step is yet to be taken. A first suggestion was that the Institute enter into a contract with the University under which the Institute has made various commitments to the University in exchange for increased financial support. As of this writing, however, a more sweeping proposal from the University is under discussion. The plan is that in exchange for its assets, the Institute will become a University-owned and supported enterprise somewhat comparable to the Center for Neural Sciences. The present Institute would then remain within the larger organization solely as a holding corporation to own highly confidential data and erotica that the University Trustees would not (care to) possess. If and when this arrangement is consummated, the Institute's present Board of Trustees will be abolished and the last vestige of the ties with the Department of Zoology will disappear.

The Institute has continued to evolve, and presently is actively pursuing a number of projects. Under the able direction of Paul G. Gebhard (Anthropology) it expanded and prospered. Scholars from all parts of the world come to Bloomington to study such diverse aspects of sex as sexual behavior in teen agers and Pre-Columbian/Peruvian erotic pottery. The current director June Machover Reinisch (Psychology, Ph.D. Columbia) has brought new enthusiasm and expertise to the Institute. As Torrey predicted the association of the Institute with the Zoology Department is historical. It is hard to believe that it began with the work of a systematic entomologist who was willing to face the criticism and rancor of what seemed in the beginning like half the world community.

During Torrey's tenure, the Department of Zoology at Indiana University was ranked a number of times in the top ten in the nation. After Torrey retired from administration W.R. Breneman, Frank J. Zeller, Robert W. Briggs and John W. Sinclair served ably as chairmen until in 1977 it was decided to unite the departments of Zoology, Microbiology, and Botany into the Department of Biology.

Zoology continues to play an important role in this new administrative unit. It is obvious, however, that the classical divisions of the biological sciences no longer mean a great deal. George Hudock works with a protozoan which has a colorless (chlorophyll lacking) mutant of a plant which might be considered an animal. Some other workers study animals with biochemical tools. In the future the fields of plant and animal sciences and biochemistry can be expected to be even more united.

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See also for addtional biographical material:

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MICROBIOLOGY AND MOLECULAR BIOLOGY

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ABSTRACTS

The Characterization of Epitopes of a Protective Surface Antigen of the Metacyclic Stage of *Trypanosoma cruzi*. Len M. Archer and Donald G. Dusanic, Department of Life Sciences, Indiana State University, Terre Haute, Indiana 47809.—A monoclonal antibody (4D37) to a surface antigen of *Trypanosoma cruzi* was bound to an Affi-Gel 10 (Bio-Rad) column and used to isolate the surface antigen (4D37 Ag) from crude extracts of *T. cruzi* epimastigotes. Eluates of 4D37 Ag were adjusted to pH 7.0, concentrated, dialyzed and used to immunize BALB/c mice. Spleen cells of the immunized mice were removed asceptically and fused with SP2/0-Ag 14 mouse myeloma cells. Forty-six percent of the wells produced hybridoma colonies of which 6 were positive when screened against crude *T. cruzi* epimastigote antigen by the ELISA. The hybridoma cells were cloned and characterized to determine antibody class and subclass. Epitope mapping studies are presently in progress. Funds for the work were provided by a research grant from the Indiana Academy of Science and NIH Grant AI 14642.

Construction of a Hybrid Plasmid Capable of Transforming Both *Escherichia coli* and *Cyanobacterium, Aphanocapsa* 6803. In Soo Bae and Carolyn N. Vann, Ball State University, Muncie, Indiana 47306.—Cyanobacteria provide an ideal system for studying both the structure and function of photosynthesis. Cyanobacteria carry out oxygenic photosynthesis just as eukaryotic chloroplast do and its simple structure allows for easy gene manipulation. *Aphanocapsa* 6803 is particularly interesting, for it is capable of not only autotrophic growth in light, but also heterotrophic growth on glucose, thus photosynthesis mutations that may be created will not be lethal. A hybrid vector which has a strong temperature sensitive promotor and is capable of controlled gene expression is being constructed. The gene encoding chlorophenicol acetyl-transferase will be inserted into one of the multiple cloning sites of the hybrid vector and the regulated expression of the enzyme will be quantified. Particular proteins involved in the assembly or function of the photosynthetic apparatus may be cloned into this vector to study the effects of site-specific mutagenesis.

Characterization of a Cytochrome P450 Deficient Mutant of *Candida albicans*. M. Bard, N.D. Lees, F.W. Kleinhans, R. Barbuch and D. Sanglard, Departments of Biology and Physics, IUPUI, Indianapolis, Indiana 46223; Merrell Dow Phar-

maceuticals, Inc., Indianapolis, Indiana 46268 and Department of Microbiology and Molecular Genetics, University of Cincinnati, Cincinnati, Ohio 45267.—A previously described *Candida albicans* nystatin resistant mutant blocked in 14α-demethylation of lanosterol was shown to also lack all traces of cytochrome P450 as determined by carbon monoxide difference spectra. This strain does not require ergosterol for growth and reverted to an ergosterol producing cytochrome P450 containing strain indicating no other lesions. The results of electron spin resonance studies indicated that the P450 lesion results in a cytoplasmic membrane that is more rigid than that of the wild type strain. Cytochrome P450 mutants described in *Saccharomyces cerevisiae* are auxotrophic for ergosterol or contain a second mutation in 5,6 desaturation of the sterol B ring. These results suggest that a cytochrome P450 lesion in these yeasts have different phenotypes and may reflect different sterol requirements for the two organisms.

Cloning of the Yeast Sterolmethyltransferase Gene. D.M. Copple, M. Bard and R.F. Gaber, Department of Biology, IUPUI, Indianapolis, Indiana 46223; and Department of Biochemistry, Molecular and Cell Biology, Northwestern University, Evanston, Illinois 60201.—Ergosterol functions as the major membrane sterol in yeast, and also provides a "sparking" function in the cell division cycle, acting as a hormone. In the ergosterol biosynthesis pathway, the methylation of zymosterol by sterol methyl transferase (SMT) is a step unique to yeast. The yeast strain used to clone the SMT gene has a mutation in the SMT gene, but may be "leaky", producing enough SMT gene product to provide the sparking function. By using gene disruption and replacement techniques, any "leakiness" of the gene will be ruled out. Five independent clones containing the SMT gene have been obtained. A restriction map of one clone will be presented. Should this methylation step prove to be required, an inhibitor of SMT could possibly serve as a fungicide.

A Mycological Assessment of Commercial Hot Tubs in Delaware and Madison Counties, Indiana. larry T. Crump, Donald A. Hendrickson, Department of Biology, Ball State University, Muncie, Indiana 47306.—A mycological analysis of several commercial hot tubs in Delaware and Madison Counties was conducted between January and April 1987. This assessment was designed to determine if a statistical relationship existed between the presence of pathogenic fungi isolated from these facilities and coliform or fluorescent pseudomonad bacteria that might be isolated from the same recreational pools. A comparison also was made between various physical parameters and the presence of fungi. Those parameters monitored included pH, temperature and residual chlorine levels. Additionally, bather loads at each facility were recorded for further comparison. Samples were taken from tubs used exclusively by males and females and, in one instance, from a facility used by both sexes concurrently. Male and female patrons used two of the unisex facilities on an alternating schedule. Samples from these tubs were taken in accordance with this schedule. A number of common fungal contaminants were isolated, as were several medically important species, including *Epidermophyton floccosum, Trichophyton rubrum, T. mentagrophytes, Candida tropicalis* and *Phialophora jeanselmei.*

Effects of Ribavirin on Rat Kidney Cells Transformed by Temperature-sensitive Mutants of Rous Sarcoma Virus. K.K. Hoopengardner, G.J. Merkel and Y.C. Chen, Department of Biological Sciences, Indiana University-Purdue University at Fort Wayne, Fort Wayne, Indiana 46805-1499.—The effects of ribavirin (1-B-D-Ribofuranosyl-1,2,4,-Triazole-3-Carboxamide), a synthetic nucleoside, on a series of morphological and growth characteristics of transformed state was studied, using rat kidney cells trans-

formed by temperature-sensitive mutants of Rous sarcoma virus. In the presence of ribavirin at permissive temperature (33 °C), cellular morphology changed from transformed phenotype to normal phenotype. This was indicated by reduction in saturation density, decreased colony formation in soft agar, and reduced hexose uptake in less dense cultures. In addition, we have also demonstrated that vimentin, an intermediate filaments protein of the cytoskeleton, is concentrated at peripheral surface of the cell membrane at the permissive temperature (33 °C), whereas at nonpermissive temperature (38 °C) it appears to be evenly distributed throughout the cell cytoplasm. Ribavirin did not appear to have an effect on vimentin distribution at 38 °C but may have caused a more diffuse pattern at 33 °C. Currently research is underway in our laboratory to attempt to quantitate the vimentin in transformed and nontransformed cells.

Analysis of Cowpea Mottle Virus RNA. JONG W. KIM, GOPI PODILA AND ROBERT F. BOZARTH. Department of Life Sciences, Indiana State University, Terre Haute, Indiana 47809.—Cowpea Mottle Virus (CMeV) is an isometric plant virus. It has a genomic ssRNA of ($^+$) polarity with mol. wt. of 1.4 x 10° daltons, and one capsid protein with a mol. wt. of 44 K daltons. Three dsRNA segments were obtained from CMeV-infected cowpea leaves. The CMeV, ssRNA and dsRNA were translated *in vitro* in a messenger dependent reticulocyte lysate. Maximum incorporation of ^{35}S-methionine into proteins was obtained at 1.15 mM Mg^{++}, 50.7 mK K^+, and 1 ug of CMeV RNA when incubated 30 min at 32 °C. Four major proteins with mol. wts. of 50, 42, 27 and 14 K were produced. The 42 K protein was selectively immunoprecipitated with CMeV antiserum. Comparative peptide mapping confirmed that the 42 K protein is CMeV capsid protein. The function of other products is unknown.

Comparison of Viruses from Healthy and Diseased Mushrooms. YOUNG H. KIM AND ROBERT F. BOZARTH, Department of Life Sciences, Indiana State University, Terre Haute, Indiana 47809.—Virus was extracted from healthy mushrooms and mushrooms of the same clone (M8) showing symptoms of the La France disease. Preprations made at pH 6.0 or 7.0 gave higher virus yields than those made at pH 8.0. Both preparations had 30 nm virus particles. Analysis of the capsid polypeptides of SDS-gels showed that the virus capsids were distinct. Virus extracts from the La France diseased samples contained nine distinct bands of dsRNA on 1.0% agarose gels which ranged from 3.5 to 0.8 kbp, whereas extracts from the VLPs of healthy mushrooms had a single band of 2.3 kbp. This band did not hybridize with the bands from the La France virus. This data clearly shows that the virus found in healthy isolates of this clone is distinctly different from the virus found in diseased sporophores of the same clone.

The Phenotypic Rescue of an Interferon Sensitive Mutant of Mengovirus, *is*-1, by Mouse L-cell Lysates. ROBERT W. KING AND EDWARD SIMON, Department of Biological Sciences, Purdue University, West Lafayette, Indiana 47907.—Interferon is an efficient antiviral protein produced by cells in response to virus infection. Viruses differ in their response to interferon. Vesicular stomatitis virus and the enterovirus (rhinovirus, poliovirus and mengovirus) are quite sensitive to the antiviral state induced by interferon whereas herpes virus, vaccinia virus, and adenovirus are resistant. In 1976, Dr. Simon *et al.*, isolated a mutant mengovirus, *is*-1, that under the proper conditions is 100-1000 times more sensitive to the effects of interferon than the wild type, is^+. When interferon-treated mouse L-cells are coinfected with is^+ and *is*-1, is^+ has the ability to increase the yield of *is*-1 to normal titers. We have found that the virus-free cell lysates prepared from is^+ infected L-cells also rescued *is*-1 in

interferon-treated cells. Thus, *is*$^+$ must produce a diffusable agent that actively overcomes the antiviral state induced by interferon and rescues the *is*-1 phenotype.

Phospholipase Production in Morphological Variants of *Candida albicans*. THOMAS LANE AND J. R. GARCIA, Department of Biology, Ball State University, Muncie, Indiana 47306.—The yeast *Candida albicans* is considered a dangerous opportunist in a compromised host. Its pathogenicity is thought to lie primarily in its ability to grow as budding yeasts or hyphae. However, this fact alone does not seem sufficient to account for its success as a pathogen. Recently, Dr. David Soll (Univ. of Iowa) described a spontaneously occurring, high-frequency switching system, in seven morphological variants which may help explain its success. We obtained some of these variants and tested them for their ability to produce extracellular phospholipase, a generally accepted mechanism of pathogenesis in many microorganisms. Our goal was to show a correlation between the amount of enzyme produced and the virulence of the variants. Using egg yolk agar plates, we showed that all variants produced the enzyme. However, one produced significantly more than the others. In all cases we insured that the variant phenotype was still expressed. Although preliminary in nature this suggests that the high-frequency switching system may modulate the virulence of the organism. Virulence tests are being conducted with Balb c mice.

Purification of Argininosuccinate Lyase (AL) by High Pressure Immuno-affinity Chromatography on an Anti-AL-silica Support. LARRY R. MASSOM, CORINNE E. ULBRIGHT, ROBERT L. SHEPARD AND HARRY W. JARRETT, III, Department of Biology, Indiana University-Purdue University at Indianapolis, and the Veterans Administration Medical Center, Indianapolis, Indiana 46223.—Argininosuccinate lyase (AL) catalyses a vital step in the urea cycle but is difficult to purify for further study. To facilitate the purification, a monoclonal antibody directed against beef AL (Anti-AL) was isolated from lymphocyte culture media by affinity chromatography on a Protein A-silica column. The purified Anti-AL was then covalently coupled to 500 A pore, 5 μm beaded 3-glycidyloxypropyl-silica and packed in a 4.6 x 30 mm column. This column was used to isolate active AL from crude liver extracts in less than 15 minutes by high pressure (performance) immuno-affinity chromatography. An alternative purification was also carried out: crude liver extract was mixed with anti-AL and applied to the Protein A-silica column. Only the AL/Anti-AL complex was retained by the column and subsequently eluted.

The Use of a Hybrid Cloning Vector for Site Directed Mutagenesis of Cytochrome B559 in *Anacystis nidulans*. BETSY A. READ AND CAROLYN N. VANN, Ball State University, Muncie, Indiana 47306.—A method for engineering site specific mutagenesis in cyanobacterium *Anacystis nidulans* R2 SPc based on the use of hybrid cloning vector is being applied for the elucidation of the function of a specific PSII protein, cytochrome b559. The shuttle vector pROAN 1 has been constructed which contains an *Escherichia coli* origin of replication, a short portion of the small indigenous plasmid of *A. nidulans* and carries both the left and rightward promoters of lambda, in addition to the gene encoding the CI857 lambda temperature-sensitive repressor protein. During modification to increase the stability of pROAN 1, a specific and unique *Bam* HI cloning site has been created directly behind the lambda promoter. The promoter-less gene for cytochrome b559 has been cloned into pROAN 1 and regulated expression is being examined. Highly conserved sequences of b559 are being altered using oligonucleotide directed metagenesis and the structure and function of the newly constructed protein is being assessed.

The Effects of Liposome Encapsulated Ethidium Bromide on the *in vitro* Growth of *Trypanosoma musculi*. SRI S. SRISKANDA AND DONALD G. DUSANIC, Department of Life Sciences, Indiana State University, Terre Haute, Indiana 47809.—Liposomes are artificial lipid vesicles that could be made in the laboratory using relatively simple techniques. They are useful in encapsulating many chemical substances for delivery to specific cells. In the present study ethidium bromide (EtBr) was encapsulated in phosphatidyl choline (lecithin-egg yolk derived) vesicles, also containing phosphatidyl ethanolamine and cholesterol. These liposomes along with control liposomes containing phosphate buffered saline were incubated with blood stream form trypomastigotes growing *in vitro* in tissue culture medium at 37°C. The growth pattern of the trypomastigotes was monitored in order to evaluate the effectiveness of liposome-drug preparations *in vivo*. At a concentration of 0.045 mg/ml EtBr the encapsulated drug killed all the trypanosomes within 72 h compared to 120 h for the unencapsulated drug. These studies were supported in part by PHS Grant AI 14642.

Differential Effects of Anacardic Acid on *in vivo* Antibody Response, Concanavalin A and Lipopolysaccharide Mitogenesis. DIANA STORHOFF AND NANCY BEHFOROUZ, Ball State University, Muncie, Indiana 47306.—Anacardic acid, the primary component of cashew nut oil extracted from the fruit shell of the Anacardium occidentale tree, and/or its alkali salts possess antipyretic, bactericidal, vermicidal, anthelminthic, antiparasitic, larvicidal, antiprotozoic activities. This study illustrates the effects of anacardic acid on the mitogenic stimulation of murine T and B cell populations by Concanavalin A (Con A) and lipopolysaccharide (LPS), respectively, and the *in vivo* antibody response of murine lymphocytes. C57/black mice subcutaneously injected with anacardic acid for 16 days exhibited enhanced mitogenic stimulation of lymphocytes by both Con A and LPS. C57/black mice subcutaneously injected for 16 days with anacardic acid and stimulated by antigen on day 15 exhibited a dose related suppression of antibody plaque formation.

Inhibition of an *in vitro* Antibody Response by Cyclosporine A. DIANA STORHOFF AND NANCY BEHFOROUZ, Ball State University, Muncie, Indiana 47306.—Cyclosporine A (CsA) is a relatively new immunosuppressive drug which has novel, poorly understood, effects on the immune system. This study attempts to elucidate the effect Cyclosporine A has on the *in vitro* antibody response of mouse lymphocytes towards sheep red blood cells (SRBC). CsA was shown to inhibit antibody plaque formation of naive spleenocytes or T and B cell mixtures when added to cultures on day 0, 1 and 2 following antigenic stimulation but not on day 3. Lymphocytes taken from animals pre-stimulated with sub-immunogenic doses of SRBC could be inhibited completely by CsA only on day 0 of culture. IL-2 rich Concanavalin A stimulated rat spleen supernatants were not able to restore the ability of CsA inhibited lymphocytes to produce antibody to SRBC antigen.

Immunological Parameters in Cyclosporine Protected Balb/c Mice Infected with *Leishmania major*. WILLIAM WHITE AND NANCY BEHFOROUZ, Ball State University, Muncie, Indiana 47306.—Paradoxically, the highly susceptible Balb/c mouse strain can be made to become resistant to the parasite pathogen *Leishmania major* by prophylactic treatment with Cyclosporine A, an immunosuppressive undecapeptide. In order to better understand the apparent immuno-modulatory effect which Cyclosporine A has on the outcome of this infection in Balb/c mice, several immune parameters were followed during infection with *L. major* in animals either treated or not treated with the drug. Mitogen responsiveness, antigen-specific antibody levels and lymphokine

production was assessed at various time intervals during infection in both groups. Results indicate that overall the immune system is suppressed only minimally early after treatment but this minimal alteration apparently leads to a profound difference in disease outcome.

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Alteration of a Cyanobacterial Hybrid Vector

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Introduction

The cyanobacteria are prokaryotes capable of performing oxygenic photosynthesis in a manner similarly observed in the chloroplasts of higher plants. Their retention of many important bacterial properties make them amenable to genetic studies. Some of these properties include: single-cell organization, single small genome, rapid generation time, transformation ability, relatively simple production of mutants, and existence of innate plasmids useful as cloning vectors. Thus, cyanobacteria are a simple model system for investigating eukaryotic-type photosynthesis (Stanier and Cohen-Bazire, 1977).

The experimental organism, *Anacystis nidulans* R2, has been well characterized biochemically and biophysically (Olson et al., 1971; Pakrasi, et al., 1985). *A. nidulans* R2 has two indigenous plasmids, an 8 kb plasmid (pUH24) and a 50 kb plasmid (pUH25) (van den Hondel et al., 1979). The smaller cyanobacterial plasmid has successfully been cloned into bacterial plasmids to create hybrid vectors that can be steadily maintained and autonomously replicated in both organisms (Golden and Sherman 1983; Kuhlemier et al. 1981, 1983; Sherman et al. 1984).

In our lab, cyanobacterial hybrid cloning vectors have been constructed for two purposes. They can be used to introduce specifically altered genes into an organism for the purposes of creating photosynthesis mutants which may be analyzed to clarify structural and functional relationships between the polypeptides involved in photosynthesis. Alternatively, they may be used to increase the expression of inserted DNA.

One such vector, pPGV5, constructed by Vann et al., 1986, contains the entire indigenous plasmid, pUH24, of *A. nidulans* ligated to plasmid pLC28 of *Escherichia coli* (Remaut et al., 1981). Plasmid pUH24 is 8.0 kb in size and contains a single *Bam*HI cloning site. Plasmid pLC28 is 2.8 kb and contains the powerful leftward promoter of the bacteriophage lambda, which allows for increased expression of a gene cloned downstream of the promoter. It also contains a gene coding for ampicillin resistance and a single *Bam*H1 cloning site. The hybrid plasmid vector constructed by the ligation of the two *Bam*HI digested plasmids is therefore 10.8 kb and has two *Bam*HI sites (Figure 1).

Plasmid pPGV5 transforms *A. nidulans* efficiently, yielding 2.7 x 10^7 transformants per microgram (Vann et al., 1986). This vector was also used to obtain some increased expression of a 36 kDa polypeptide involved in iron regulation in iron deficient medium (Vann et al. 1986).

Although plasmid pPGV5 showed promise of allowing enhanced expression of cloned genes, these experiments were postponed until the plasmid could be altered to create a more efficient cloning vector. It was altered by removing approximately half of the cyanobacterial sequences to produce a smaller vector capable of more efficient transformations in *E. coli*. The *Bam*HI cloning site most distant from the lambda phage promoter was also removed to create a unique cloning site. The altered cyanobacterial hybrid vector, pTNTV, which is described here is 6.8 kb and contains a single *Bam*HI cloning site conveniently located downstream of the lambda phage promoter.

Materials and Methods

Construction of plasmid pTNTV is depicted in Figure 1. In order to construct this plasmid, two restriction endonuclease digestions were needed: a complete digestion with endonuclease *Xho*I was required to insure that all molecules were cut at

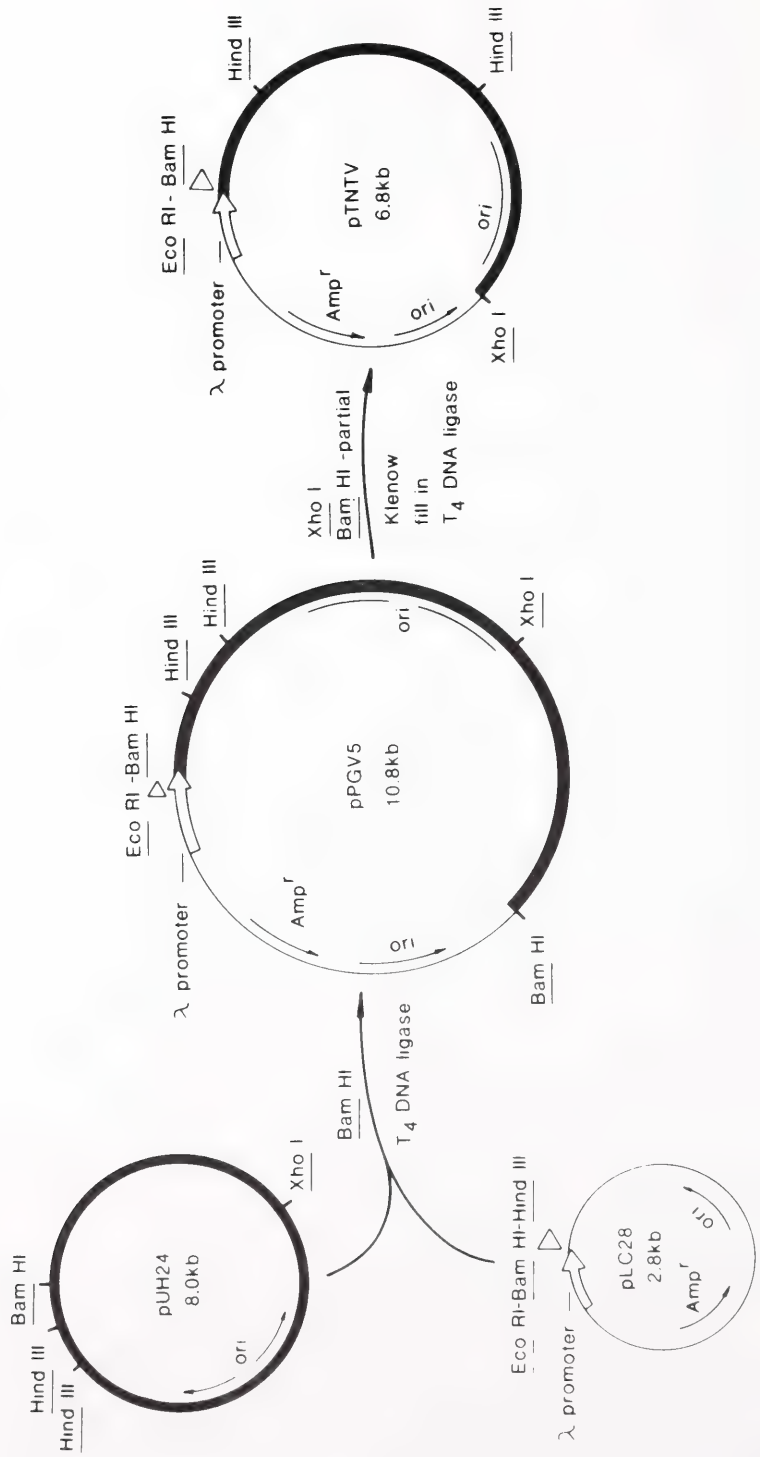

FIGURE 1. Construction of plasmid pTNTV.

this site and a partial digestion with *Bam*HI which would result in some molecules cut only at the *Bam*HI site most distant from the lambda promoter.

First, plasmid PGV5 was first digested with the restriction endonuclease *Xho*I. The digestion was carried out in four separate reaction tubes each containing 3 ug of plasmid DNA in a 15 ul solution of 25 mM Tris-HCL (pH 7.8), 50 mM NaCl, 10 mM $MgCl_2$, 100 ug/ml BSA, 2mM beta-mercaptoethanol, and 5 units of *Xho*I. After incubating at 37 C for 1 hour, the partial digestions with *Bam*HI were performed. The salt concentrations in tubes 1-4 were adjusted to 100 mM NaCl and 5 units of *Bam*HI was added to tube 1. The concentration of *Bam*HI in tube 1 was thus 1.6 units/ug DNA. Serial dilutions of the endonuclease were made by removing 5 ul of the reaction mixture from tube 1 and tube 2. The enzyme concentration in tube 2 had then been diluted to 0.41 units/ug DNA. This fourfold serial dilution was continued through tube 4, transfering 5 ul each time. Digests were incubated 15 minutes at 37 C. The digests were stopped by the addition of phenol. A phenol/chloroform extraction was performed followed by an ethanol precipitation.

Each sample was resuspended in 15 ul of 10 mM Tris-HCL, 1 MM EDTA (pH 8.0) and analyzed by gel electrophores through a 0.7% agarose gel. It was determined that the digestion conditions in tubes 3 and 4 were optimal, yielding a large number of molecules cut twice. These tubes were then pooled and the Klenow fragment from *E. coli* DNA Polymerse I was used to fill-in the restriction enzyme-induced 3' recessed ends. The reaction was carried out using 2 ug of the restriction fragment in a 25 ul solution containing a 2 mM mixture of all four deoxynucleotides, 0.5 M Tris-HCl (pH 7.2), 0.1 M $MgSO_4$, 1 mM dithiothreitol, 100 ug/ml BSA, and 2 units of Klenow. This reaction was incubated at 22 C for 15-30 minutes.

A ligation of the blunt end fragment was then carried out in the same reaction mixture with the addition of 2 units of T4 DNA ligase. After an additional 6 hour incubation at 22 C, the ligation volume was increased to 100 ul with 10 mM Tris, 1 mM EDTA pH 7.6.

Thirty microliters of the ligation mixture was used to transform *E. coli* HB101 using standard techniques (Maniatis 1982). Plasmid DNA was isolated from transformants (Maniatis 1982), and confirmation of plasmid TNTV was obtained via restriction analyses using *Eco* RI, *Hin*dIII and *Bam*HI.

Results and Discussion

The new hybrid cloning vector, pTNTV, was constructed as described above. In Figure 2 it can be seen that this vector is smaller (approximately 6.8 kb) than pPGV5 (10.8 kb) and that pTNTV contains only one *Bam*HI restriction site as compared to pPGV5 which has two. Digestions with *Hin*dIII proved that the new vector retains the cyanobacterial fragment which carries the origin of replication (data not shown). Experiments are in progress to determine if pTNTV will retain the high level of transformation efficiency observed with plasmid pPGV5.

There are several ways that the new vector, pTNTV, can be beneficial. It can be used to explore the effects of enhanced expression of specific wild-type and mutant photosynthesis genes. This is a useful approach for elucidating the possible functions, reactions, and structural relationships of particular proteins involved in the photosynthetic process. The vector may also be used to examine high levels of expression of heterologous genes such as those from higher plants.

Currently we are initiating a project to examine the use of this expression vector as a means to control malaria which is transmitted to humans by the bite of *Anopheles* mosquitoes (dipteran insects). *Bacillus thuringiensis* is a spore-forming bacterium that produces protein inclusions that are lethal to select groups of insects. We have obtained a gene from a strain of *B. thuringiensis* which encodes a dipteran-specific

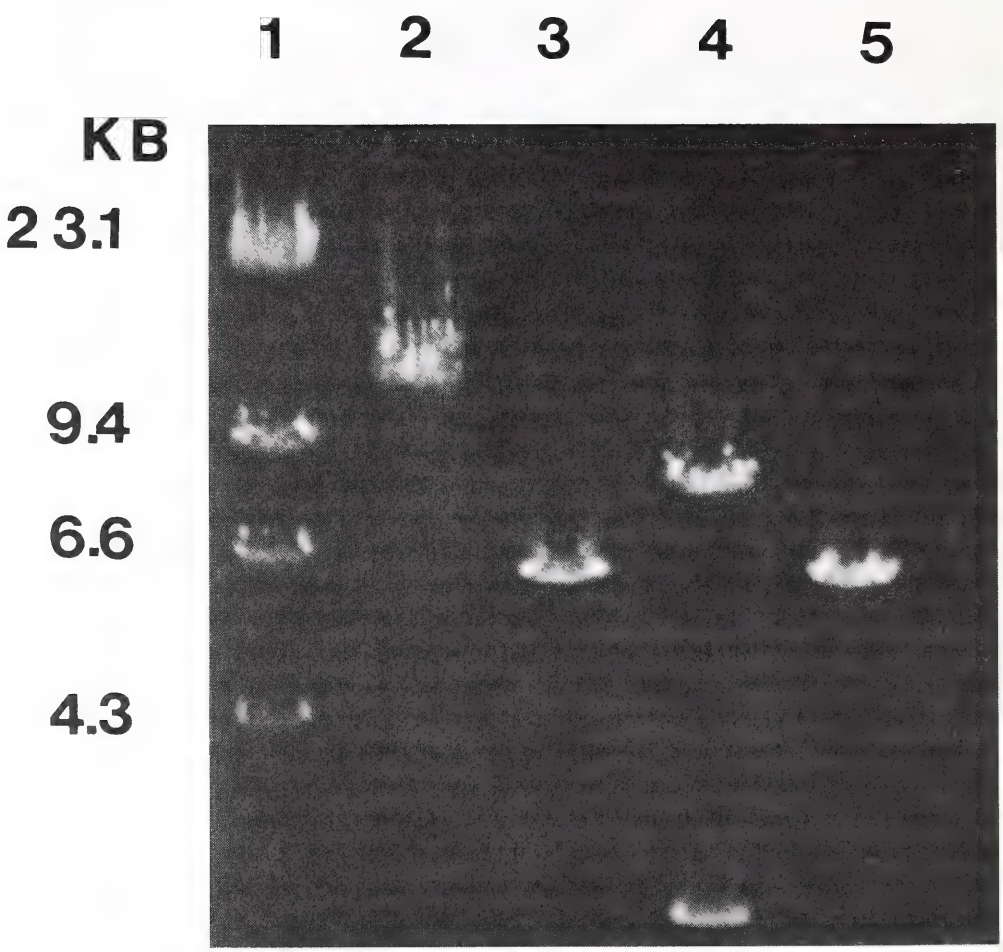

FIGURE 2. Agarose gel electrophoresis demonstrating the construction of plasmid pTNTV. Lane 1 contains the molecular weight markers, bacteriophage lambda digested with endonuclease *Hin*dIII. Lanes 2 and 4 contain plasmid pPGV5 digested with *Eco*RI and *Bam*HI, respectively. Lanes 3 and 5 contain plasmid pTNTV digested with *Eco*RI and *Bam*HI, respectively.

insecticidal protein. We are currently cloning this gene into pTNTV and will then transform *A. nidulans*. If the cyanobacteria survive and a high level of toxin expression is observed, laboratory studies will be performed in which mosquito larvae will be reared with the transformed cyanobacteria. Thus, a more effective method of control of malaria-carrying mosquitos may be obtained.

In conclusion, a new hybrid vector has been constructed which allows for a high level of expression of a gene cloned downstream of the powerful lambda promoter. The vector has two unique cloning sites downstream of the promoter, *Bam*HI and *Eco*RI. The small size of this vector will allow for efficient transformation into *E. coli* or *A. nidulans* R2. Thus, the vector, pTNTV, may prove to be a powerful tool in investigations of the effects of high levels of expression of homologous or heterologous genes in cyanobacteria.

Acknowledgment

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The Mechanism of Retinoic Acid Mediated Effects on Cell Growth and Differentiation

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Introduction

Retinoids (vitamin A) elicit many biological responses from cells both *in vivo* and *in vitro*. They have been shown to display distinct roles in differentiation of embryonal carcinoma (27,28), melanoma (20) and choriocarcinoma (5) cells *in vitro*. Additionally, they cause chemoprevention of cancer (2,10,14,26). It is generally believed that the biological action of retinoids is mediated by cellular retinoid binding proteins. Furthermore, a retinoic acid-binding protein in plasma membrane has also been described (7,25).

Relatively little is known about the mechanism of retinoid-induced cell differentiation. Induction of differentiation of human promyelocytic HL60 cells by retinoids, accompanied by an increase in the intracellular pH, has been described (19). Subsequently, the induction of NAD+-glycohydrolase (NADase) activity of a leukemia cell line and two human monoblast cell lines has been demonstrated (15). Recently, changes in intermediary purine metabolism which appeared to contribute to the regulation of terminal maturation in cells have also been reported (21). The concept that production of guanosine nucleotides and the activity of inosine monophosphate dehydrogenase (IMD) have an important role in regulating cell differentiation has been proposed (22). Finally, an association with growth arrest has been observed during retinoic acid-induced differentiation of F9 cells (9).

A temperature sensitive simian virus 40 (SV40) transformed fetal rat hepatocyte line (*RLA209-15*) exhibits a normal, differentiated phenotype when grown at 40 °C. However, the maintenance of the transformed status is observed when grown at 33 °C (4). Previous reports indicated that SV40 transformed mouse fibroblast cells were less sensitive to retinoic acid (17). Therefore, the *RLA209-15* cell line provides a more suitable model system to study the action mechanism of retinoids.

In this study we examined the effects of retinoic acid on cell growth, cell morphology and transmembrane electron transport activities of cells. The inhibition of growth and transmembrane electron transport activities is associated with a decrease in NAD+ pool which can lead to a fall of GTP formation (1,21). It has been proposed that a hypothetical cytoplasmic inducer for differentiation might exist in an uninduced state by binding to GTP (E. S. Golub, personal communication). According to this hypothesis, the fall of GTP concentration causes the dissociation of GTP from the inducer and allows the derepressed inducer to activate specific genes and therefore induce cell differentiation.

Materials and Methods

RLA209-15 fetal liver cells were cultured in α-modified minimal essential medium (α MEM, from Irvine Scientific Company, with arginine, supplemented with 0.4 mM orinthine), 100 μg/ml streptomycin, and 100 u./ml penicillin plus 4% fetal bovine serum and gassed with 5% CO_2 and 95% air as described previously (Chou and Schlegel-Haueter, 1981). After 3 days of culture at 33 °C, some cells were cultured for 2 more days at 40 °C to suppress transformation and cause a return to normal phenotype. The rest of cells remained at 33 °C to maintain the transformed phenotype.

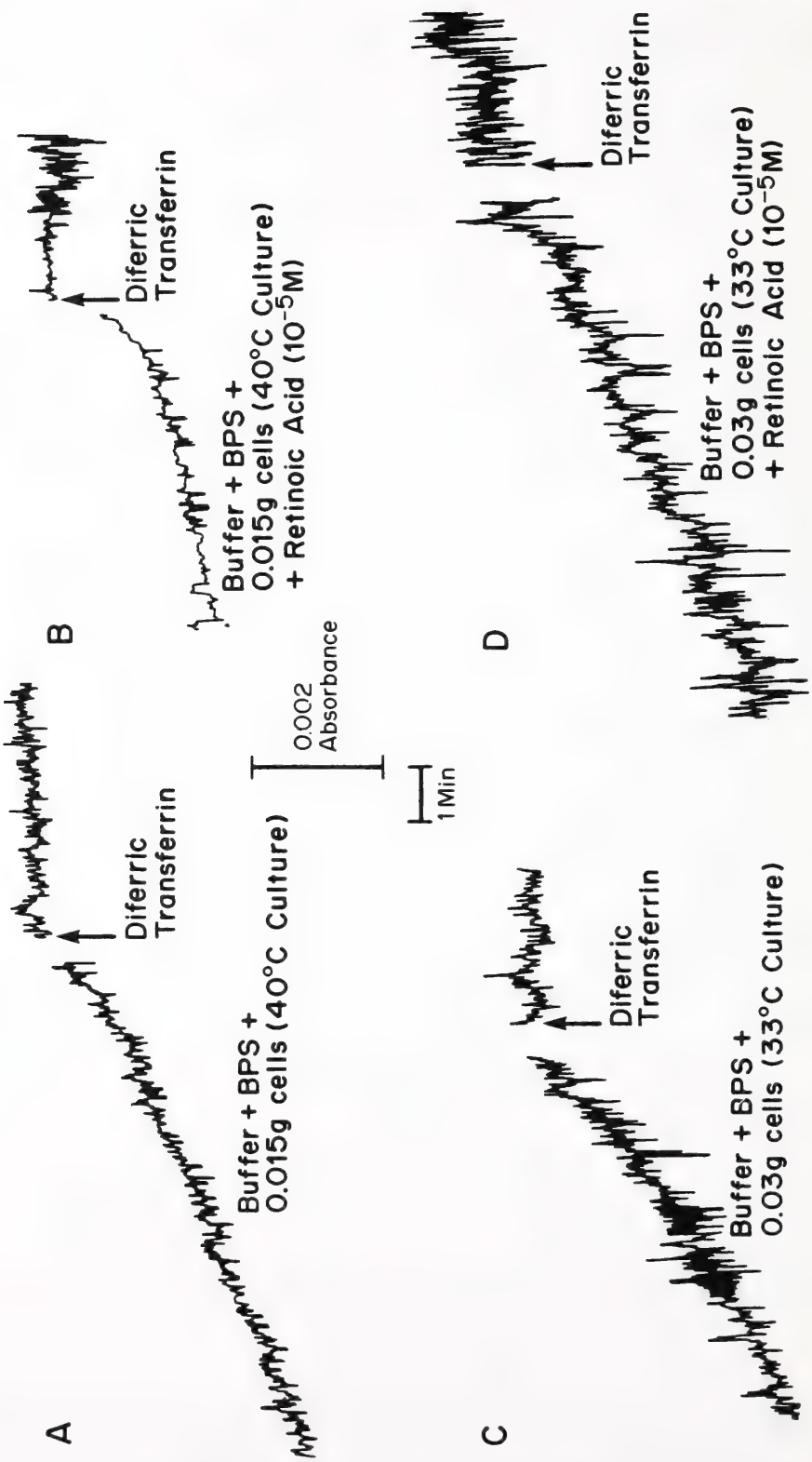

FIGURE 1. Spectrophotometer tracings of diferric transferrin reduction by transformed (33 °C) and non-transformed (40 °C) *RLA209-15* rat fetal liver cells. (A) 40 °C cells without retinoic acid; (B) 40 °C cells with retinoic acid (10^{-5}M); (C) 33 °C cells without retinoic acid and (D) 33 °C cells with retinoic acid (10^{-5}M). The reaction was run from right to left on the chart.

Cells were then harvested in the same media and washed in salts-Tris-EDTA buffer (140 mM NaCl, 2.5 mM KCl, 0.6 mM Na_2HPO_4, 25 mM Trizma base, and 0.05 mM EDTA, pH 7.4) (TD buffer). The pellet was resuspended in the same buffer to a final concentration of 0.1 gram wet weight per ml (gww/ml).

The effect of retinoids on cell growth was examined by growing cells in various concentrations of serum with and without retinoids. After 2 days of incubation at 37 °C, cells were harvested and a cell survival count was taken immediately. Survival was determined by an eosin Y exclusion method as described by Mitchell and Shrigi (24).

Assay of transmembrane electron transport activities was performed by measuring the reduction of iron in diferric transferrin (Fe_2Tf). The formation of ferrous bathophenanthroline sulfonate was determined according to Avron and Shavit (3). Absorbance change at 535 nm was subtracted from absorbance at 600 nm with the dual beam on the DW2a Aminco spectrophotometer. Extinction coefficient difference is 17.1 mM^{-1} cm^{-1}. The assay mixture in 2.8 ml TD buffer contained 3.4 μM diferric transferrin, 3.3 μM bathophenanthroline sulfonate and 0.015 gww of cells. Diferric transferrin with an absorbance ratio over 0.04 at 465:280 nm was prepared according to Karen and Mintz (18) or obtained from Miles Laboratories. Apotransferrin, used to prepare diferric transferrin, was from Sigma. Assay temperature was 37°.

The pyridine nucleotide pool was determined by extraction of NAD or NADH from cells with perchloric acid and alkali, respectively, as described previously (16). The extracted pyridine nucleotide was then quantitated using a cycling assay involving alcohol dehydrogenase (23).

Plasma membrane (PM) was isolated from rat liver as described by Goldenberg *et al.* (13). NADH diferric transferrin reductase activity with isolated PM (0.02-0.1 mg protein) was measured as described (33) or by following the oxidation of NADH (50 μM) in the presence of Fe_2Tf (3.4 μM) and 1 μM of KCN in 2.8 ml of 50 mM phosphate buffer (pH 7.2).

Results

The reduction of Fe_2Tf by cells is measured by the formation of a chelate, ferrous bathophenanthroline sulfonate in the external media since bathophenanthroline sulfonate is impermeable to cells. The spectrophotometer tracings of Fe_2Tf reduction by transformed (33 °C) and on-transformed (40 °C) *RLA209-15* rat fetal liver cells with and without all trans retinoic acid is shown in Figure 1. Retinoic acid does not inhibit the reduction rate by 33 °C transformed cells (Figure 2 and Figure 1D), however, a 67% inhibition of the reduction rate is observed with cells cultured at 40 °C (Figure 2 and Figure 1B). The inhibition is specific for all trans retinoic acid and is not observed with other retinoids such as retinol and retinyl acetate (Table 1).

RLA209-15 (40 °C) rat fetal liver cells are known to exhibit a differentiated phenotype (4). The alteration in the expression of liver functional proteins in the presence of retinoid has also been described (6). Most recently, the induction of F9 cell differentiation by retinoids accompanied by a growth arrest was reported (9). We therefore examined the growth effect of retinoids on these cells. Growth inhibition caused by retinoic acid is shown in 40° cultures only but not in 33 °C cultures as indicated by the effect of retinoic acid on cell growth (Table 2). It should be noted in this context that growth stimulation as a result of the activation of transmembrane electron transport activities (11,29,31) and growth inhibition due to the inhibition of this activity by drugs (8,30) has been demonstrated. Since retinoic acid selectivity interferes with Fe_2Tf reduction of the normal phenotype and not of the transformed cells, it is not surprising that retinoic acid causes growth arrest of the normal cells but has no effect on growth of the transformed cells. A correlated pattern of inhibition

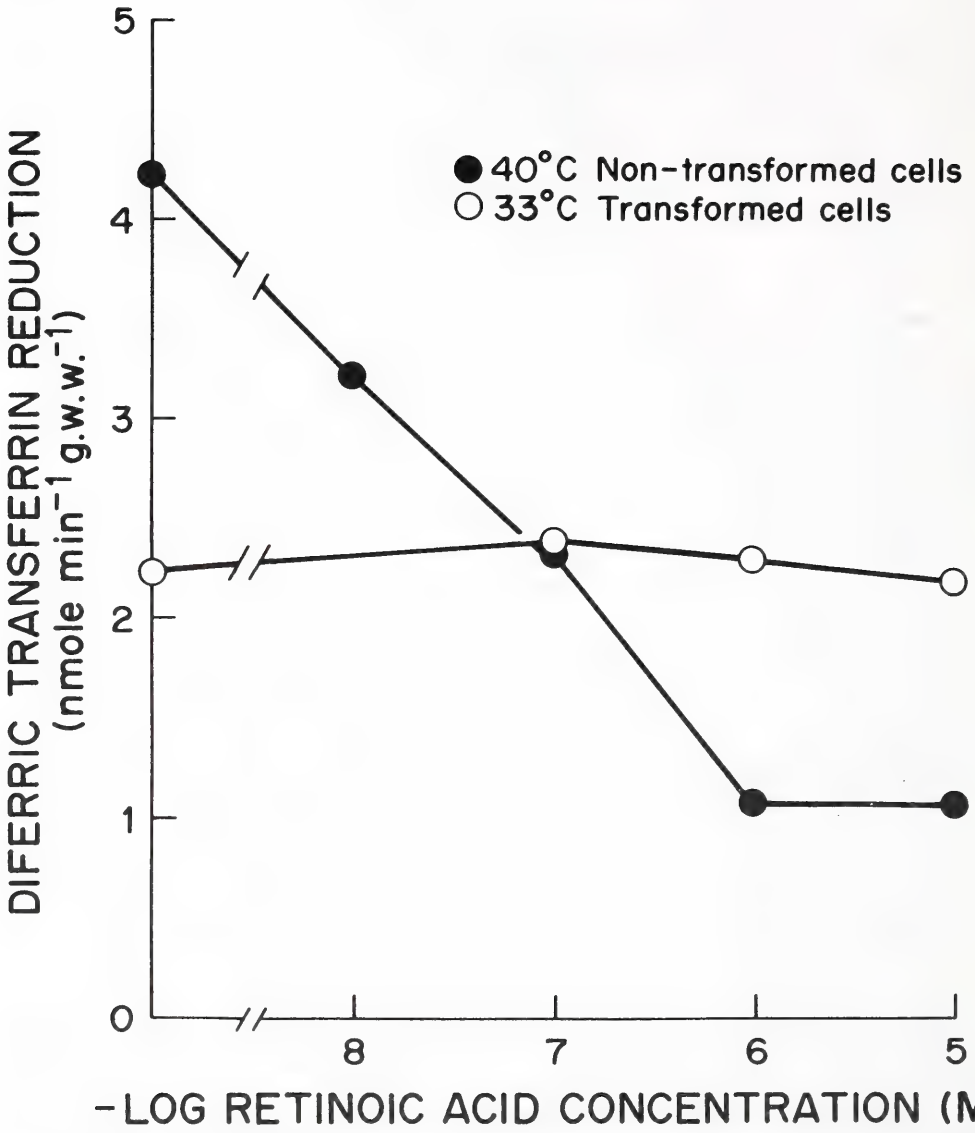

FIGURE 2. Effect of retinoic acid on diferric transferrin reduction by *RLA209-15*. Cells were grown in 33 °C and 40 °C incubator separately for 48 hr. Cells were fed fresh media containing retinoic acid (concentration as indicated on Fig. 1) daily.

TABLE 1. The Effect of Some Retinoids on the Reduction of Diferric Transferrin by Fetal Rat Liver Cells Infected with a Temperature Sensitve SV40 Virus (*RLA209-15* Cells).

Compounds	Fe_2Tf Reduction (nmoles $min^{-1}gww^{-1}$)	
	40 °C cells	33 °C cells
None	5.7	2.2
Retinoic Acid (10^{-6}M)	2.8	2.3
Retinoic Acid (10^{-5}M)	0.2	2.3
Retinol (10^{-6}M)	4.4	2.2
Retinol (10^{-5}M)	4.1	1.9
Retinyl Acetate (10^{-6}M)	4.8	--
Retinyl Acetate (10^{-5}M)	3.0	1.4

Representative of three different experiments with different batches of cells for retinoic acid inhibition.

TABLE 2. Effect of Retinoic Acid on the Growth of Fetal Rat Liver Cells Infected with a Temperature Sensitive SV40 Virus (*RLA209-15* Cells).

Calf Serum Volume Percent	Number of cells ($4\ 10^{-5}$) / 25 cm² flask			
	40°C Cells		33°C Cells	
	Without RA	With RA (10^{-5})	Without RA	With RA (10^{-5}M)
0	1.13	0.71	1.37	1.41
2.5	4.04	2.93	3.64	2.87
5	4.15	3.04	5.15	5.58
7.5	4.17	3.27	5.41	5.56
10	4.43	3.25	5.68	6.25

RA = retinoic acid

of Fe_2Tf reduction and growth inhibition in response to retinoic acid is shown between 40°C and 33°C cultures. The morphological study also shows that retinoic acid induces giant cell formation in 40°C cultures but no significant morphological changes in 33°C cultures (Figure 3). There is no morphological differences between the untreated cultures of 33°C cells and 40°C cells.

The consequence of the inhibition of electron transport should be a decrease in NAD^+. Therefore, the NAD^+ concentratiuon in 40°C and 33°C cells was determined at various times after treatment with retinoic acid. The results are seen in Table 3. It can be seen that the NAD^+ levels in 40°C cultures decrease nearly 50% whereas 33°C cultures show an insignificant change over the 36 hour period of treatment. The reduction rate of Fe_2Tf was also compared with the same batch of cells. A correlated inhibitory pattern is seen between NAD^+ level and Fe_2Tf reduction in

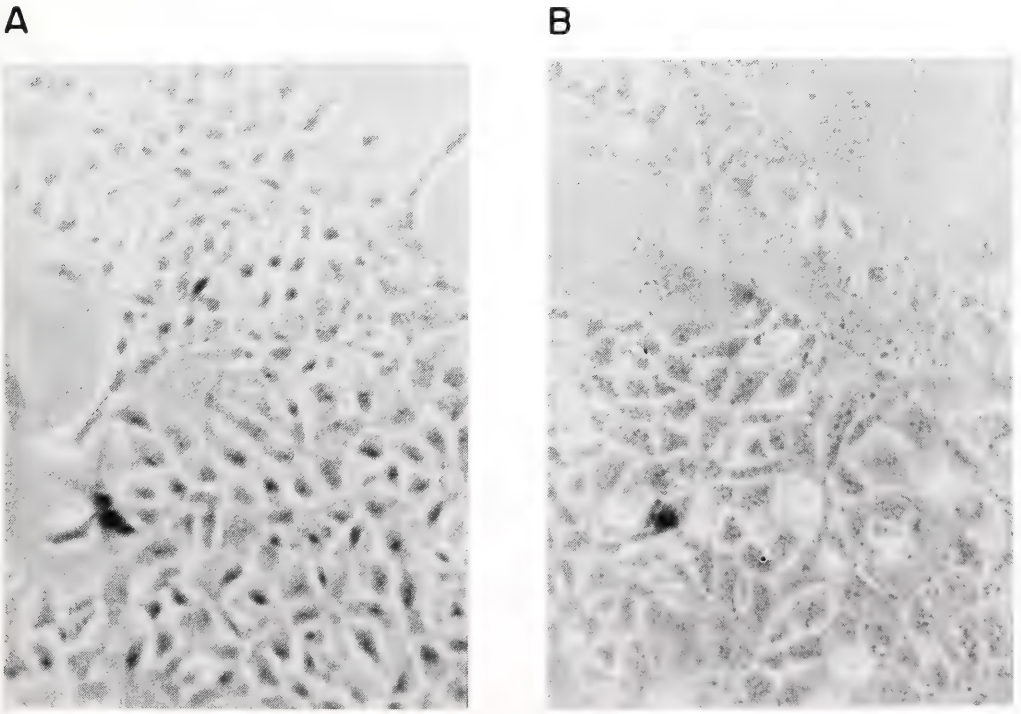

FIGURE 3. Effect of retinoic acid on the morphology of 33°C cultures and 40°C cultures. (A) 33°C cultures; (B) 40°C cultures. Cells were grown in media containing retinoic acid (10^{-5}M) for four consecutive days.

TABLE 3. Effect of Retinoic Acid on NAD+ Level and Diferric Transferrin Reduction with Fetal Rat Liver Cells Infected with Temperature Sensitive SV40 Virus (*RLA209-15* Cells).

Hour of Treatment	NAD+ (pmole/mg protein)		Fe_2Tf Reduction (nmole $min^{-1}gww^{-1}$)	
	40°C Cells	33°C Cells	40°C Cells	33°C cells
0	110.5	38.2	20.7	9.0
6	96.6	39.6	16.2	9.1
12	69.1	35.5	10.9	9.0
24	55.1	36.3	5.2	9.1
36	52.4	33.9	4.1	5.7

Concentration of retinoic acid used in the experiment is 1×10^{-5}M. The SV40 virus is expressed in the cells cultured at 33°C but not in the cells at 40°C.

40°C cells. Essentially, the reduction rate of Fe_2Tf is not changed in 33°C cells over 24 hours.

The effect of retinoic acid was also tested on NADH-diferric transferrin reductase activity of the purified rat liver plasma membrane. The assay can be performed either by measuring NADH oxidation or by measuring the formation of ferrous bathophenanthroline chelate after the addition of Fe_2Tf. The results are shown in Table 4. Both assay methods show a strong inhibition of enzyme activity by retinotic acid. We therefore believe that the mechanism for retinoic acid action begins at the plasma membrane.

Discussion

The evidence that transplasma-membrane redox systems are closely related to cell growth and development has been demonstrated previously (8, 11, 29-31). It is reasonable to consider that retinoic acid may inhibit cell growth due to its effect on redox systems. The variation in sensitivity to retinoic acid between 40°C cell cultures and 33°C cultures in this study is similar to differences in sensitivity to retinoic acid observed with mouse fibroblast cells (3T3 cells versus virus-transformed 3T3 SV cells) (17) in which an inhibition of exponential cell growth and a reduction in saturation density were compared.

Since we have shown that retinoic acid inhibits the redox system in purified plasma membrane from rat liver the site of action of retinoic acid can be at the

TABLE 4. Effect of Retinoic Acid on NADH-Diferric Transferrin Reductase of Rat Liver Plasma Membrane.

Assay Procedure for Measuring Reductase Activity	Addition	% of Control Activity
Ferrous-bethophenanthroline chelate formation	None (control)	100
	RA (10^{-7}M)	50
	RA (10^{-6}M)	29
	RA (10^{-5}M)	13
NADH oxidation stimulated by diferric transferrin	None	100
	RA (10^{-7}M)	62
	RA (10^{-6}M)	45
	RA (10^{-5}M)	42

RA = retinoic acid.

membrane (32). This is consistent with the fact that retinoic acid can induce cell differentiation even when immobilized outside the cell (34).

According to Chou's study (4), the temperature-sensitive rat fetal liver cell line, *RLA209-15* exhibited a normal differentiated phenotype characterized by increased levels of both α-fetoprotein (AFP) and albumin when grown at the nonpermissive temperature 40°C (4). The ratio of these functional liver proteins was altered, and the biosynthesis of the polypeptide chain of AFP was modified during treatment with retinoids (6). However, the synthesis of both functional proteins was inhibited following transformation by growing cells at the permissive temperature (33°C). In addition to these differences, our results indicate an alteration of the NAD^+ pool of non-transformed cells, but not of transformed cells, following the treatment with retinoic acid. The decrease in the NAD^+ pool of 40°C cultures can be a consequence of the inhibition of electron transport activity. The fall in NAD^+ level could act to down regulate inosine monophosphate dehydrogenase (IMPD), a key enzyme in purine biosynthesis. IMPD is NAD^+-dependent and very sensitive to changes of concentration of NAD^+ (1) and therefore a fall in GTP level can be a result of a decrease in NAD^+ (E. S. Golub, personal communication).

Studies in microbial systems showed inhibition of IMPD induced differentiation (sporulation) in both *B. subtilis* and yeast (12). It has been postulated that the fall in GTP derepresses an inducer molecule which then binds to methylated DNA and results in new gene transcription (E. S. Golub, personal communication). Additionally, Lucas and his coworkers showed with mammalian systems that a fall in GTP level is a first consequence of retinoic acid induction of differentiation (21). Based on these reports plus our studies, we postulate that retinoic acid reacts with a receptor molecule or a binding protein in the membrane which then reacts with a cytoplasmic inducer molecule which is in the uninduced state due to the repression by being bound to GTP. The dissociation of GTP from the inducer takes place when the fall of GTP occurs and therefore allows the now derepressed inducer to activate the specific genes and induce cell differentiation.

Since both our transformed and non-transformed cells are derived from the same clone of *RLA209-15* cells, the different response should not be due to differences in the uptake, metabolism and intracellular fate of retinoids in the cells. This system provides a useful tool for examining the mechanism of retinoic acid action in differentiation.

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PHYSICS AND ASTRONOMY

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ABSTRACTS

A Feasibility Study for the Detection of Radon in Water by the Liquid Scintillation Method. Alice S. Bennett, Cheryl A. Bye, Hsin-I-Kao, David R. Ober and Michele M. Vakili, Ball State University, Muncie, Indiana 47306.—A Beekman LS 3801 liquid scintillation counting system was used to determine radon concentration levels in drinking water. Samples were prepared by introducing 10 ml of sample into the glass counting vial under 5 ml of scintillator flour. Calibration of the system was achieved by preparing Rn-222 standards from Ra-226 source solutions.

Radon concentration levels were determined for several wells and sources in east-central Indiana. One site was monitored daily for a period of two weeks in an attempt to establish baseline data for local fluctuations. In a separate study the activities for two samples were determined daily for sufficient periods of time to confirm that the characteristic 3.82-day half life associated with Rn-222 was being observed.

Real-time Holographic Interferometry of Vibrating Structures. Richard Booe and Uwe J. Hansen, Department of Physics, Indiana State University, Terre Haute, Indiana 47809.—The ability of critical replacement of the hologram in the exposure location to within a fraction of a wavelength of the recording light opens the possibility of observing interference between the reconstruction of the holographic image of the undeformed object with the light reflected from the displaced surface of the object. Generally the contrast between interference fringes for vibrating systems under such circumstances is insufficient for critical study. This study reports an attempt to increase that contrast by pulsing the laser illumination at the frequency of object excitation.

Optical Analysis of a Reflecting Strip Solar Concentrator. Clarence Brooks and Ronald Cosby, Department of Physics and Astronomy, Ball State University, Muncie, Indiana 47306.—Fresnel mirrors using flat reflective strips constitute one possible method for reducing the cost of reflective-type solar concentrators. In this project, the design and performance of a perfectly tracking, line-focusing concentrator is studied using an optical ray trace analysis. For constant width mirror strips mounted on a flat base, strip tilt angles and positions are determined for sunlight concentration on a flat finite-width absorber located at a selected focal position. Computation of the intensity distribution of concentrated sunlight in the absorber plane is described.

Solar Food Drying in the Republic of Guinea. Alseyni Diallo and Ronald Cosby, Department of Physics and Astronomy, Ball State University, Muncie, Indiana 47306.—Drying is an important method of food preservation in the tropical Republic of Guinea, especially for the dietary staple fish. Primary methods for fish drying range from the expensive use of charcoal and other fuels to open air solar drying. The use of solar food dryers to dry fish economically, rapidly and in a protected environment is proposed. Load considerations are reviewed and include the moisture diffusion processes and drying stages present. A solar dryer design is presented and progress on a dryer analysis is discussed.

Initial Studies of Radon Gas at Mammoth Cave National Park Using Activated Carbon Canisters. Vincent A. DiNoto, Jr., Jefferson Community College Southwest, Louisville, Kentucky 40272 and John Swez, Indiana State University, Terre Haute, Indiana 47809.—Radon gas (Rn-222) has been monitored at Mammoth Cave National Park since the middle 1970s. The monitoring has been done on a weekly basis using high volume air samplers and alpha particle detectors. The method used is the Kusnetz method. The levels measured within Mammoth Cave vary throughout the year and are dependent upon the temperature and pressure of the air outside the cave. These two meteorological parameters cause a change in the flow patterns of the air within the cave system. The levels which have been measured have been between 0.5 and 1.2 working levels.

During the spring and summer of 1987, we began a monitoring program of the cave system using activated carbon canisters. The canisters were counted on a multichannel analyzer with a NaI photomultiplier. Gamma rays of the Radon daughters were measured. The initial results of this data will be discussed.

Contributions of the Ford Foundation to Astronomy. Frank K. Edmondson, Department of Astronomy, Indiana University, Bloomington, Indiana 47405.—"Who have made the most important contributions to Astronomy?" Harlow Shapley answered this in the 1930s by naming Andrew Carnegie and John D. Rockefeller. The Carnegie Institution of Washington had built and supported the Mount Wilson Observatory, and the Rockefeller Foundation had funded the construction of the 200-inch Palomar telescope. The name of Henry Ford should be added to this list because the Ford Foundation provided funds in the 1960s for four important astronomical projects in the southern hemisphere: 1) The European Southern Observatory (ESO), $1,000,000, 1959 (paid 1964); 2) The Yale-Columbia Astrograph in Argentina, $750,000, 1960; 3) CSIRO for the Australian Solar Radio Telescope (Radioheliograph), ($550,000 plus a supplement of $80,000) = $630,000, 1962 & 1966; 4) Association of Universities for Research in Astronomy, Inc. (AURA) for half the cost of the Cerro Tololo (Chile) 150-inch telescope, $5,000,000, 1967.

These four grants will be discussed with special attention to the first and last, ESO and AURA.

An Effective Lagrangian for the Bosonic Sector of the Standard Model with a Heavy Fermion. Eduardo V. Flores, Department of Physics, Indiana State University, Terre Haute, Indiana 47809.—This paper is a study of the effects of a one-fermion family with a heavy particle in the low-energy boson sector of the Standard Model. In this approach, one of the fermion masses is light (m) and the other is heavy (M). At the one-loop level, heavy-fermion mass effects proportional to M^2 and $\ln(M/m)$ have been found. The form of the structures present in the effective Lagrangian has been

found by studying the symmetries of the path integral in the presence of a heavy fermion. For the sake of manageability, only the case of a heavy Higgs boson will be illustrated.

The Physics of Steel Drums. UWE J. HANSEN, Department of Physics, Indiana State University, Terre Haute, Indiana 47809; THOMAS D. ROSSING AND SCOTT HAMPTON, Department of Physics, Northern Illinois University, DeKalb, Illinois 60115.—The normal modes of vibration of a musical instrument contribute significantly to the radiated sound. The boundary conditions associated with individual sections of a steel drum, along with the inherent stiffness of the steel are conducive to considerable coupling between sections. The inherent normal modes of steel drum sections along with their relative coupling between sections has been studied using holographic interferometry and impulse excitation modal analysis. Results of the two techniques will be compared.

Pedagogical Advantages of SDI. R.H. HOWES, Department of Physics and Astronomy, Ball State University, Muncie, Indiana 47306.—Examples drawn from the technologies of SDI, popularly known as Star Wars, illustrate simple principles of physics such as the conservation laws. Although the physics and mathematics involved in these examples are very basic, they demonstrate some of the major technical issues involved in the debate over the feasibility of space based defenses against nuclear missiles. Real-world examples dealing with current issues show beginning physics students that even algebra-based physics can provide quantitative and relevant results on politically important problems. Students begin to dare to apply common sense estimates and back-of-the-envelope calculations using basic physics to problems drawn from the world around them. At the same time, better students become interested in learning the more advanced physics behind the sophisticated weapons described in these simple examples.

Air Resistance of the Ball-and-string Pendulum. M. MCINERNEY, Rose-Hulman Institute, Terre Haute, Indiana 47803.—The air resistance of a simple pendulum consisting of a golf ball supported by a nylon fishing line was studied experimentally by varying the length and width of the fishing line. With this technique we were able to distinguish between the air resistance due to the ball and that due to the line. Resistance terms linear and quadratic in speed were found and compared with theory. We found that the linear air resistance of the nylon line varied with width in contradiction to theory; and that the quadratic resistance of the golf ball was larger (at low speeds) than that of a smooth sphere in agreement with the observations of others. This work was supported in part by a grant from the Indiana Academy of Science.

An Orthogonal Least Squares Method for Data Correlation. BENJAMIN P. MILLER AND HOWARD E. DUNN, University of Southern Indiana, Evansville, Indiana 47712.—An orthogonal least squares method was developed for linear correlations of data sets in which both variables are subject to uncertainties in measurement. The method is based on expressing a standard linear regression solution in a rotated coordinate frame. The angle of rotation is determined for this line fit to satisfy the orthogonal least squares condition. The solution was found to be sensitive to the relative numerical scales of the two variables, but its application is considered appropriate to data sets rescaled to a particular dimensionless form which gives equal weight to both variables. A versatile computer program was developed for application of the method.

Determination of Indoor Radon Concentration Levels by Detecting Daughter Decay Products with a Geiger Counter. DAVID R. OBER AND CLIFFORD M. ROTENBERG, Department of Physics and Astronomy, Ball State University, Muncie, Indiana 47306.—Air samples were drawn through 0.8-micron Millipore filters (AAWP 047 00) at a rate of 20 liter/minute for 15 minutes. The air filter was placed between two facing GM tubes and count rates were obtained for 10-15 successive one-minute intervals. Count rates obtained by this method were compared to daughter concentration levels of radon and radon daughters measured by gamma-ray/high volume air sampler and canister methods, respectively. This simpler sampling and counting procedure was sensitive to radon levels at 0.5 pCi/liter; the procedure was found to be portable, quick, inexpensive, and quite suitable for detecting relative variations in concentration levels of radon daughters.

Physics of Concentrator Solar Cells. MOMTAZ SHAHEEN AND RONALD COSBY, Department of Physics and Astronomy, Ball State University, Muncie, Indiana 47306.—Solar cells used with solar concentrators are exposed to varying and high levels of illumination. For dispersive concentrators, the concentrated sunlight intensity and its spectral content are non-uniform across the cell. Thus, P-N junction theory used as part of a concentrator solar cell model must consider high level injection and an appropriate absorption model. In this paper, a simple model of an N+-*P*-*P*+ concentrator solar cell is reviewed and extensions considered. Solutions of the continuity equations for excess carriers are presented and cell current-voltage relations are given.

Demonstration of the Charcoal, Cannister Method of Radon Detection. JOHN SWEZ, Department of Physics, Indiana State University, Terre Haute, Indiana 47809.—The charcoal absorption technique is now very widely used to measure indoor radon concentrations. The technique requires exposing activated charcoal within a metal cannister for a period of two to seven days in an area of suspected radon concentration. After this passive period, the charcoal is counted for gamma rays emitting from radon daughters. A laboratory experiment demonstrating this procedure will be conducted. Different methods of evaluating radon daughter concentrations, the influence of changing exposure conditions such as relative humidity, radioactive daughter decay calculations and various techniques of calibration will also be briefly mentioned. Handouts will be provided so that the audience can easily understand the methodology involved.

Solution of Maxwell-Lorentz Equations in Co-moving Coordinate Systems. JAMES B. WESTGARD, Department of Physics, Indiana State University, Terre Haute, Indiana 47809.—The general solution to the charged particle equations of motion is particularly elegant in a co-moving coordinate system. By defining a velocity field (representing a set of initial conditions) the equation becomes a particularly simple, partical differential equation. The calculations are performed in covariant form throughout, and the solution is seen to be related to conservation of canonical momentum. Examples of solutions for simple cases are given.

Measurement of Atmospheric Turbidity in Central Indiana. BRUCE WILCOXEN AND WALTER CARNAHAN, Department of Physics, Indiana State University, Terre Haute, Indiana 47809.—We report the measurement of atmospheric turbidities in the area around Indianapolis. The turbidities have been measured by means of a sunphotometer calibrated on Mt. Evans. The turbidities have been measured at five wavelengths. Four of these wavelengths are coincident with the first four Thematic Mapper bands; the fifth is the World Meteorlogical Organization standards at 500 nm. The spectral information is analyzed to determine the particulate size distribution.

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Historical Supernovae

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Abstract

Throughout recorded history, records of unusual and interesting astronomical events have been made with varying degrees of reliability. The earliest observations of comets date back to the 23rd century B.C., but with doubtful authenticity. The first reliable record of a comet was in the year 1095 B.C. The class of objects known as supernovae, visible to the naked eye, have only eight reliable recorded events. The last event took place on February 23, 1987. The other seven verified supernovae occurred in the years AD 185, 393, 1006, 1054, 1181, 1572 and 1604. An historical/scientific discussion of these eight super-explosions will be presented which will identify the characteristics of this exclusive category of astronomical objects.

Introduction

Early views of the universe were typically centered on the belief that the sea of visible stars was constant and unchanging—the Aristotelian view of celestial perfection. The human eye as a sensor can detect less than 10,000 of the millions of known stars in our universe. Optical aids were not available until the early 1600s and thus the view of the universe was very limited—with few exceptions. These exceptions were "new" stars originally dubbed *nova*. This paper deals with the correlation of historical records with actual data from the remnant of a given explosion.

In 1885 a new star was discovered near the center of the Andromeda galaxy (M31) by Ernst Hartwig at the Dorpat Observatory in Russia. Heber D. Curtis recognized the importance of this new star which was brighter than all other known novae. This discovery started the search for other, very bright novae. In 1934, Walter Baade and Fritz Zwicky presented a classic paper in which a new term was introduced into the field of astronomy—*supernova*. (The 1885 event in the Andromeda galaxy was the first supernova detected outside the Milky Way galaxy and now about a dozen are seen (*not* naked eye) each year in other galaxies.) They plotted the number of novae occurring in nearby galaxies within the past hundred years versus their absolute magnitudes. The resultant histogram showed a distinct bimodal grouping—the more numerous fainter group (absolute magnitudes $+13$ to $+16$) and a brighter group (absolute magnitudes $+7$ to $+10$). The histogram showed us events in the absolute magnitude range $+10$ to $+13$. Those in the brighter group were dubbed the "supernovae" to distinguish them from those in the fainter "novae" group.

Supernovae Types

Novae and supernovae are two types of objects listed in the General Catalogue of Variable Stars as eruptive stars. While the eruptive event is taking place, a history of the variation in the amount of light versus time can be made. This light signature is known as a *light curve*. The two most common types of supernovae light curves are known as Types I and II, as shown (1) in Figures 1 and 2. Type I supernovae are characterized by one major outburst of light followed by a gradual exponential decay (typically lasting up to 300 days from the time of the initial outburst). These

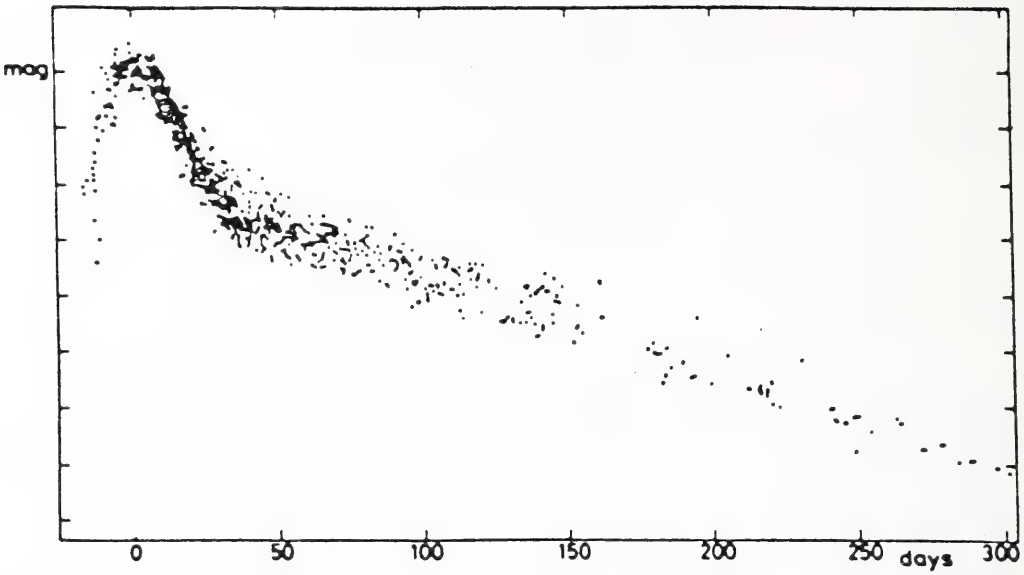

FIGURE 1. Composite light curve from 38 Type I supernovae.

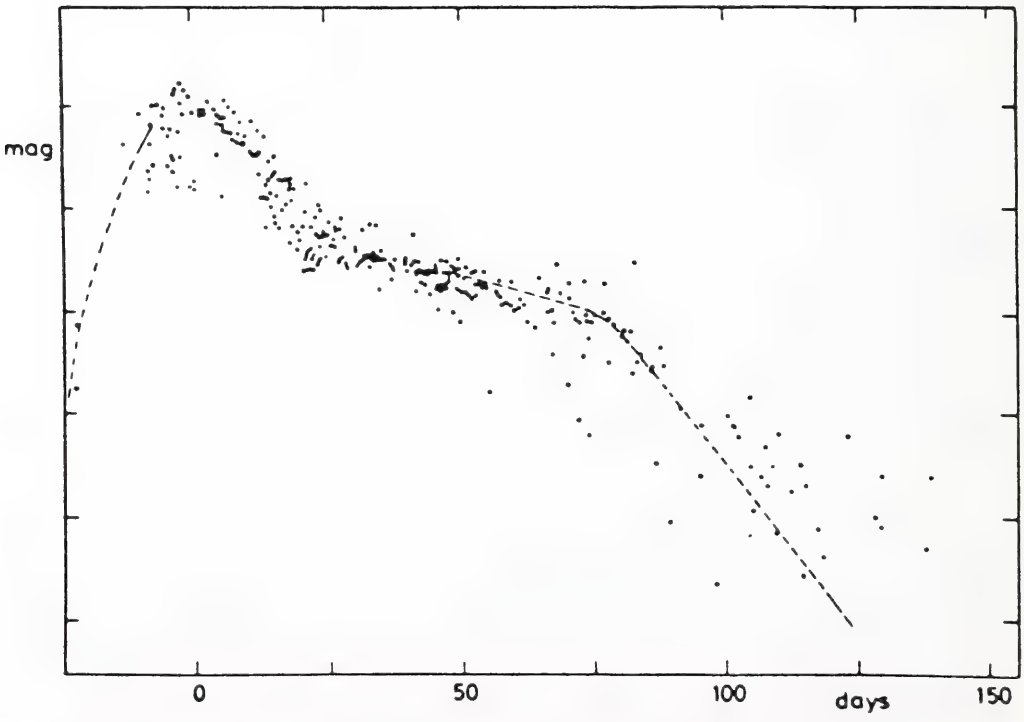

FIGURE 2. Composite light curve from 13 Type II supernovae.

events are thought to arise in double star systems where one component pulls material from the other, thus making it unstable. Type II supernovae are characterized by an initial outburst of light which is not as violent but with several secondary outbursts for the first 75 days followed by a very rapid decrease in the light output. The vertical axes of both figures are in magnitudes, in which the scale varies from brightest (top) to faintest (bottom). In addition to their light variation signatures, the two types of supernovae vary in another important aspect. The spectra of these events show that the Type II supernovae have strong emission lines of hydrogen (Balmer

lines) and strong absorption lines of ionized calcium. The Type I supernovae do not have emission lines of hydrogen but do show strong absorption lines of ionized silicon and iron. Type I supernovae occur in all galaxy types whereas Type II supernovae are confined to galaxies that show strong evidence for ongoing star formation (e.g., the arms of spiral galaxies).

Supernovae Remnants (SNRs)

The four principal observable remnants of a supernova are (1) a pulsar, (2) an expanding optical nebulosity, (3) a region of radio emission and (4) an extended X-ray source. Very few SNRs exhibit all four remnant types but the region of radio emission appears to be common to all events. The intensity of the radio emission increases with increasing wavelength—non-thermal radiation caused by the synchrotron mechanism. In a synchroton, electrons are accelerated to very high speeds in a magnetic field. In a supernova, the electrons of low energy emit radiation at radio wavelengths and exhibit a high degree of polarization, a property verified in data from SNRs.

Source of Historical Records and Candidates

Historical records of astronomical events with enough information to reconstruct and locate the events are mainly from three countries in the far east—China, Japan and Korea. The main source of data is from the Chinese and is limited to the year 213 BC and later, for it was in that year that the "burning of the books" took place by direction of the emperor Ch'in Shih-huang. The Han dynasty (202 BC - AD 220) established an astronomical office as part of a special subdepartment within the Ministry of State Sacrifices. This Astronomical Bureau had two major functions: (1) to maintain an accurate calendar and (2) to observe and interpret celestial portents. The official establishment of the Astronomical Bureau marked the beginning of organized acquisition of astronomical data that has survived.

The term K'o-shing was used to describe "guest stars" or "visiting stars". This term appears to be synonymous with novae and supernovae, but the term sometimes is associated with motion and thus occasionally used to describe a comet. The two thousand years of written records describing bright "guest stars" contain approximately 75 objects. However, if the restraint that only the ones visible for at least five months (thus eliminating comets) is imposed, the list is reduced to only 20 objects. These 20 then represent the historical records of the novae that were bright enough to be seen as naked-eye objects. Distribution studies of supernovae in other galaxies shows that they tend to be constrained to the galactic plane. Thus, if the further restriction of limiting the list of candidates to only those objects which lie close to the galactic plane (galactic latitude (b) less than 25 degrees) then the list is reduced to the seven events given below. the apparent magnitude (m) at maximum light and the distance to the remnant (d) in light years.

TABLE 1. Historical Supernovae Candidates.

Year	*Duration*	*b*	*m*	*d*
AD 185	20 months	15°	−4	6500
AD 393	8 months	0°	−1	—
AD 1006	several years	+15°	−10	4200
AD 1054	22 months	−5°	−5	6500
AD 1181	185 days	0°	0	28000
AD 1572	16 months	0°	−4	10000
AD 1604	12 months	+5°	−3	26000

Around AD 275 the Astronomer Royal of China estimated from early star maps that there were 283 star groups (asterisms) of 1464 stars. Later figures vary but these figures are fairly representative. The Chinese historical records relate the event with respect to the recognized asterisms. By comparison, there are today 88 recognized constellations whose boundaries are set by the International Astronomical Union.

AD 185

The event of AD 185 was reported to be "within" the asterism Nan-men, the two bright stars Alpha and Beta Centauri. A survey of this region yields four candidate (2) supernova remnants. To have a SNR correlated with the reported event, the radio, X-ray and optical evidence must yield a SNR within 5000 light years and less than 2000 years old. The SNR that lies directly on a line between Alpha and Beta Cen is *not* that SNR, but the one that lies about two degrees south of the line fulfills both criteria (and is the only one of the four candidates to do so). Thus, the remnant associated with the SN of AD 185 is RCW 86 (G315.4-2.3).

AD 393

The event of AD 393 meets all of the criteria with respect to visibility and galactic position; however, the historical records place the event in the asterism *Wei* (a portion of the constellation Scorpius) and covers an area greater than 40 square degrees. This area has been surveyed a number of times and there are seven known (3) remnants "within" this asterism. Unfortunately, because the asterism covers so large an area, any unambiguous SNR identification is not possible. Since it was observed for such a long period (eight months), it was almost certainly a supernova, but the other half of the puzzle cannot be completed with any certitude. Thus, this event will have to be dropped from the final list of historical supernovae.

AD 1006

The event of AD 1006 occurred in a fairly populated portion of the sky, but it also represents the brightest of the events in the historical records. This brightness was fortunate in that it provided important limits on the search area for its remnant. A record has survived from a Benedictine monastary at St. Gallen in the Alpstein mountains in Switzerland. The importance of this sighting is that it sets a southern declination limit on an otherwise fairly large search area. The southern limit is defined by the limited visibility of the monks from St. Gallen because of the restricted southern view (2). This southern limit defines the preferred search area and yields only one eligible candidate (2) — PKS 1459-41. Because candidate meets all other criteria, another case can be closed with considerable confidence.

AD 1054

One of the most famous objects in the sky is the Crab Nebula (M1). The new star in AD 1054 occurred in a very uncluttered portion of the sky near the star Zeta Taurus. The only SNR in the vicinity is the Crab Nebula (2). This is a very easy identification match and is unambiguous.

AD 1181

The event of AD 1181 represents the third supernova recorded by the Sung dynasty astronomers (AD 1006, AD 1054 and AD 1181). This event also marks the transition event—by the time of the next visible supernova in 1572, European astronomy well surpassed that of the Far East. The AD 1181 event occurred in a very cluttered region in the vicinity of the constellation Cassiopeia. Convincing evidence exists for

3C 58 being the SNR and there is no other remnant in the vicinity that could correspond. There is a great deal of confusion in the correlation of the historical records with a known SNR. The confusion results from the critical interpretation of a single word in the historical records. Thus, the correlation is listed as probable.

AD 1572

The event of AD 1572 appeared shortly after the publication of Copernicus' *De Revolutionibus Orbium Coelestium* (AD 1543). The Renaissance was well under way in Europe and scientific progress was rapid, whereas the Chinese and Korean scientific expertise was relatively unchanged over the centuries. The light from the supernova arrived at Earth during the time of the Danish astronomer Tycho Brahe (AD 1546-1601). Brahe was an extremely careful observer who recorded the position of the supernova with respect to nine reference stars in Cassiopeia and with respect to Polaris. The positional measurements of Brahe were accurate to within about one arc minute. (By comparison, many of his contemporaries recorded measurements that are accurate to only about half a degree.) Brahe's records also yield an unambiguous Type I supernova event. The SNR 3C 10 has been positively identified at optical, radio and X-ray wavelengths. The correlation of the supernova to the SNR was a much easier task with the precise positional information provided by Brahe.

AD 1604

Just 32 years after the supernova of 1572, the last pre-telescopic (astrononical use of the telescope dates from around 1609) supernova was discovered. Brahe died in AD 1601 (just three years prior to the event) but his student and successor, Johannes Kepler (Ad 1571-1630), made detailed observations of the supernova of 1604. As with SN 1572, the supernova of 1604 was a Type I event. Apparently the new star was detected almost simultaneously by astronomers in Europe and the Far East. Kepler was not the first one to detect the supernova but was the one who made precise measurements both of its position (error of less than one arc minute) and its brightness. Japanese historical records fail to include either this supernova or the one of AD 1572.

SN 1987A

On February 23, 1987 the light from a supernova that erupted 160,000 years ago reached Earth. This is the first naked-eye supernova since the event in AD 1604 and the first since the advent of telescopes. Unlike the six historical supernovae described above, this supernova occurred not in our Galaxy but in one of our neighboring galaxies—the Large Magellanic Cloud (LMC), 160,000 light years from Earth.

This event has caused supernova researchers to rethink some basic theoretical assumptions. Prior to this event the progenitor star was always thought to be a red supergiant. Identification of the progenitor star is difficult in the very cluttered region of the LMC, but it is thought that it was a blue supergiant (which is smaller than a red supergiant). This could explain why the event was not as bright as expected. The spectra distinctly show, lines of hydrogen. Thus the event is a Type II. However, the light curve in the first 20-30 days does not match other Type II light curves (mainly because it did not become as bright as expected).

Thus far, the event 1987A has produced energy at optical, ultraviolet and X-ray wavelengths. Gamma rays are expected to reach Earth in a few months. The event also led to the birth of neutrino astronomy with the recording of a neutrino burst at Earth (eight bursts were recorded in the United States and eleven in Japan). It is too soon for a SNR—but soon, another SNR will be correlated with this naked-eye supernova.

Summary

Only six events survive the rigid constraints as outlined, yielding a very probable or a certain link between the historical records and a known supernova remnant. The radio remnants correlated with these six survivors are given in Table 2. The historical records provide important data, for they place a limit on the age of the remnant and aid in identifying the correct candidate among multiple candidates in a cluttered field. A supernova is the result of an explosion of a certain type of star. The amount of energy released is typically 10^{51} SI units (equivalent to about 10^{28} one-megaton hydrogen bomb explosions). It is a fairly rare event and apparently only seven have been naked-eye objects as viewed from planet Earth.

TABLE 2. Historical SN-SNR Correlations.

SN	*Radio Remnant Identifications*
AD 185	G315.4-2.3 (RCW 86)
AD 1006	G327.6+14.5 (PKS 1459-41)
AD 1054	G184.6-5.8 (Ml)
AD 1181	G130.7+3.1 (3C 58)
AD 1572	G120.1+1.4 (3C 10)
AD 1604	G4.5+6.8 (3C 358)

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Solution of the Asymmetric Light Curve of OO Aquilae

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Abstract

The variability of the binary star system OO Aquilae was discovered by Hoffleit (6). Since that time, a number of its published photoelectric light curves show a definite asymmetry with a pronounced difference in maximum light (at elongation). The suggestion by Naqvi and Grønbech (21) of the hot region (to account for the asymmetry) on the binary star system TY Mensae, whose light curve is similar to OO Aquilae, has been incorporated into the light-curve modeling program by Nagy (18) used in the analysis of OO Aquilae. The results of this modeling with be presented and compared with solutions presented by other authors.

Introduction

The analysis procedure assumes a geometry based on the centrally condensed Roche model formulated in circular-cylindrical coordinates and second-order, limb-darkening fits to the model stellar-atmosphere calculations of Carbon and Gingerich (5). These assumptions have been applied to Lucy's model (12, 13), a common convective envelope for the W Ursae Majoris systems, and have been incorporated into the model developed by Nagy (18), which includes calculation of the reflection effect (second order). The gravity-darkening exponent (g) and the effective albedo (A) values are drawn from theoretical calculations by Lucy (11) and Rucinski (24, 25), respectively.

The light-curve synthesis procedure by Nagy (18) has been applied to the Johnson (7) V photoelectric light curve of Lafta and Grainger (10) of the W Ursae Majoris type eclipsing binary system OO Aquilae. The results of this analysis yield the relative sizes of the binary stars, their mass ratio (q) and the inclination of the orbital plane of the binary system (i). A comparison with analyses by other authors (Johnson (7) B and V bandpasses only) for photoelectric light curves at different epochs is presented.

Present Work

Tables of gravity coefficient values have been given by several authors in the past (Russell (26); Binnendijk (3) and Kopal (8)) using different assumptions at selected wavelengths. The generating expression utilizes a graybody assumption given to the first order by Kopal (8):

$$y = c_2/(4\lambda T_o\ (1-\exp(-c_2/\lambda T_o)))$$

where y is the monochromatic gravity coefficient, $c_2 = 1.438 \times 10^8$ angstroms/degree (second radiation constant), λ is the wavelength in angstroms, and T_o is the effective temperature in degrees Kelvin. Essentially, the quantity y is used to convert the Stefan bolometric surface brightness to a Planck monochromatic surface brightness. The most common bandpasses used for light curve photometry are the Johnson (7) UBVRI and Stromgren (27) ubvy systems. The effective temperatures of the hotter stars (O and B) as given by Bohm-Vitense (4) tend to be higher than those by Johnson (7), but those stars are rarely of interest in binary star light-curve modeling. The modeling program used here is fairly insensitive to small variations in the values of the monochromatic gravity coefficient and to temperatures as a function of the spectral class.

The W Ursae Majoris eclipsing binary system OO Aql is a Binnendijk (2) A-type system. A summary of the adopted astrophysical parameters for the binary system is given in Table 1. The adopted model stellar-atmosphere parameters used in the computation of the synthetic light curve are given in Table 2. The limb-darkening

TABLE 1. Adopted Physical Parameters for OO Aquilae.

Quantity	*Value*	*Reference*
spectral type (Sp)	G5V	Roman (23)
effective temperature (T_{eff}) (primary star)	5660K	Böhm-Vitense (4)
period (P) in days	0.50678914	Lafta and Grainger (10)
bolometric albedo	0.5	Ruciński (25)

coefficients are derived values from the model stellar-atmosphere grids of Carbon and Gingerich (5). These derived values result from a second-order fit of the data in a least-squares reduction of the grid data values. The monochromatic gravity coef-

TABLE 2. Adopted Model Atmosphere Parameters for OO Aql.

wavelengths (angstroms)	limb-darkening coefficients u_1	u_2
B = 4400	0.864 ± 0.004	−0.063 ± 0.003
V = 5500	0.799 ± 0.006	−0.125 ± 0.004
	monochromatic gravity coefficient (y)	
B = 4400		1.49
V = 5500		1.20

ficients are from the generating expression given above. A summary of catalogue data for the system is presented in Table 3. This table provides other catalogue identifiers, spectral type and magnitude ranges for OO Aql. The synthetic light curve shown in Figure 1 was computed at 47 normal points (plotted as asterisks). Each of the normal points represents approximately 20 individual observations.

TABLE 3. Catalogue Data for OO Aql.

Reference	*Identifier*	*Sp*	*Magnitude Range*
Wood et al. (29)	2844	G5V	8.4 - 9.2
	BD +08°4224		
	HD 187183		
	HV 5468		

The individual data points are the individual observed points of Lafta and Grainger (10) plotted as phase vs. magnitude. In the plot, the magnitude has been zero-point shifted from the published data so that maximum light is plotted at approximately zero magnitude. The zero-point adjustment was 0.80 magnitude for the V data values (e.g., this value was added to the observed data). Computer modeling of physical systems permits the modeler the option of picking from a grab bag of parameters. Great restraint must be exercised to prevent too many parameters from varying since the solution parameters then begin to lose their physical meaning. It is important also to apply physical limits to parameters (e.g., the mass ratio should be permitted only inclusive values between zero and unity). The present analysis solves

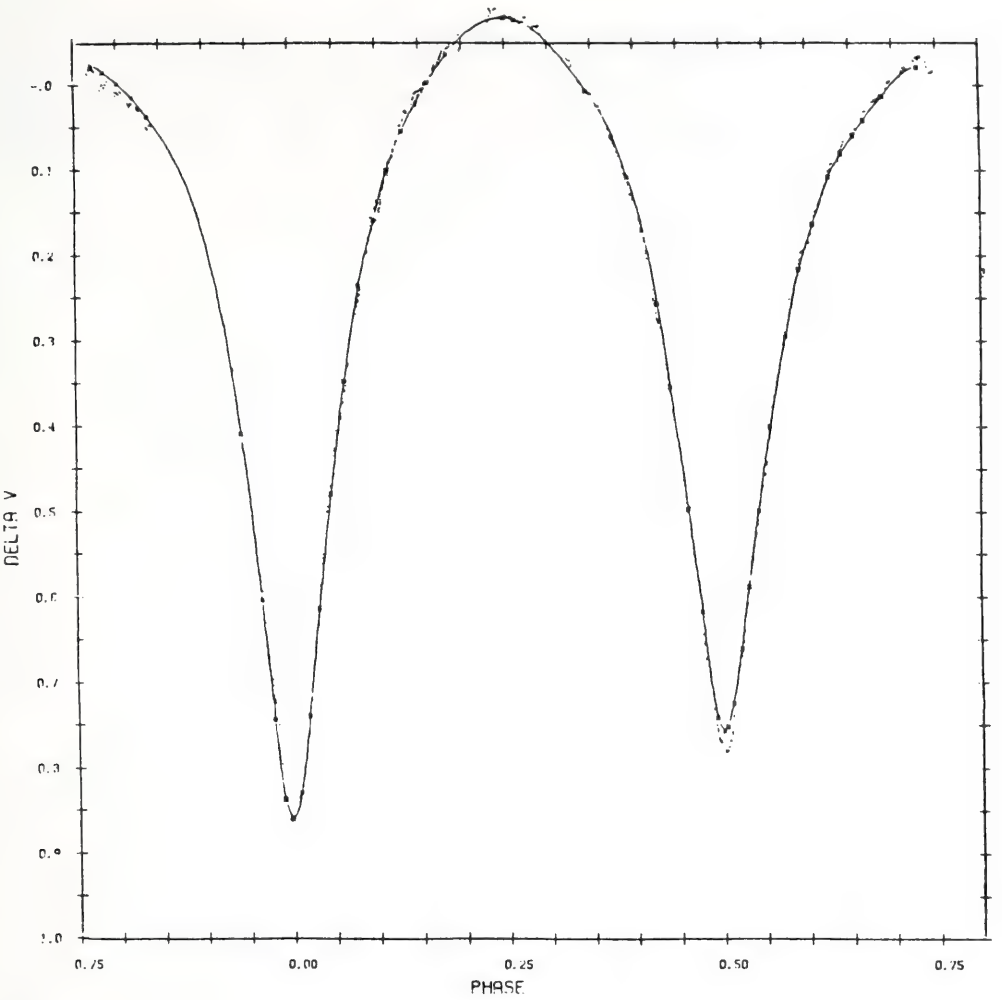

FIGURE 1. The relative V light curve of the binary star system OO Aql. The observations (small dots) are those of Lafta and Grainger (10) and the synthetic light curve has the parameters as given in Table 5. The computed values of the modeled light curve are plotted as asterisks and the solid curve is the spline fit through the computed values.

directly for three parameters: surface potential (C), inclination (i) as mass ratio (q). From these parameters a number of secondary parameters are computed (e.g., temperature differences and the relative sizes of the component stars).

Hot Spot

As shown in Figure 1, the *V* light curve of OO Aql is asymmetric as can be seen by comparing the amount of light at the two elongations of the system (i.e., phases 0.25 and 0.75). A number of the W UMa binary star systems exhibit this behavior but only recently have modeling programs been constructed to deal with it. Mullan (17) has proposed magnetic starspots to explain the asymmetry in the light curves of the W UMa class eclipsing binary stars. The presence of a hot spot (i.e., an area with a higher relative temperature than the surrounding area) on the primary manifests itself as an increasing effect from longer to shorter wavelengths (21). However, for the system OO Aql, as the wavelength decreased, the effect of the

hot spot decreased. This is opposite the effect of a hot spot on the primary, which leads to the conclusion that the hot spot is on the secondary component of the system. Table 4 summarizes the analyses of three different eclipsing binary star systems exhibiting asymmetric light curves. Two have low mass ratios, with the hot spot on the primary (AG Vir and TY Men). The other has a high mass ratio with a hot

TABLE 4. Summary Comparison of Asymmetrical W UMa Stars.

Star	AG Vir (20)	TY Men (19)	OO Aql
λ	V, B	y, b	V
Inclination	$78\overset{\circ}{.}9 \pm 0\overset{\circ}{.}3$	$76\overset{\circ}{.}9 \pm 0\overset{\circ}{.}3$	$86\overset{\circ}{.}3 \pm 0\overset{\circ}{.}2$
Mass Ratio	0.277 ± 0.005	0.211 ± 0.005	0.819 ± 0.009
Fillout	1.39 ± 0.01	1.20 ± 0.01	1.01 ± 0.01
Primary: a b c	.55 .52 .48	.56 .53 .49	.42 .39 .37
Secondary: a b c	.33 .28 .27	.29 .25 .25	.39 .36 .34
Spot latitude	$75° \leq \beta \leq 115$	$75° \leq \beta \leq 120°$	$-85° \leq \beta \leq 175°$
Area of spot	7.6%	6.7%	33.1%
T_p	7900 °K	7560 °K	5660 °K
T_s	5200 °K	6875 °K	6475 °K
Period	$0\overset{d}{.}64265$	$0\overset{d}{.}46166$	$0\overset{d}{.}50679$
Depth in Minima			
I	$0\overset{m}{.}55$	$0\overset{m}{.}48$	$0\overset{m}{.}92$
II	$0\overset{m}{.}45$	$0\overset{m}{.}40$	$0\overset{m}{.}82$

spot on the secondary component (OO Aql). Mochancki (15) has proposed that contact binaries be divided into three classes: the traditional W- and A-type systems of Binnendijk (2) and a new class the 'B-type systems', after Lucy and Wilson (14). The premise is that the B-type systems have evolved into a contact binary (evolved systems of large mass ratio and low mean density possess too much angular momentum to have existed as contact systems at age zero).

In a period study of OO Aql, Lafta and Grainger (10) found that, in a plot of frequency versus time, after about a 20 year consistency, the period starts decreasing with a sudden change around 1960. This change perhaps represents the onset of contact of the stars' the photospheres. If the trend of the plot continues, the binary system will increase its fillout, thus decreasing the period of the binary system.

Intercomparison of Analyses

A tabulated summary of the analyses of this system is given in Table 5. The Lafta and Grainger (10) analysis utilized the optimization method (a distorted spherical model). The results quoted for Niarchos (22) are based on the method of Kopal (9) which analyzes the light curves of close eclipsing binaries by transforming the data into the frequency domain. This method is quite different from the others used in the analysis of this system and hence some of the published results do not map directly into the standard set of parameters. In terms of the physical sizes, shapes of the components, mass ratio and degree of fillout for the binary system, the present results are very similar to those of Twigg (28). The Lafta and Grainger (10) solution compares favorably with present results in terms of mass ratio and inclination. Although there is similarity between the present analysis and the other solutions listed in Table 5, the difference is manifest in the parameter k, which is the ratio of the average radii of the smaller star with respect to the larger star.

The degree of fillout has been defined by Mochancki and Doughty (16) in terms of the surface potential (C), the innermost potential surface (C_1) and the outermost potential surface (C_2). When the fillout is less than unity ($O < F < 1$), the system is detached. In this case the surface potential of the system is greater than the inner-

TABLE 5. Comparison of Light Curve Analyses of the System OO Aql.

Analysis (Data)	Wynne and Nagy (10)	Lafta and Grainger (10)	Niarchos (22) (1)	Twigg (28) (1)
λ	B, V	B, V	B, V	V
q	0.819 ± 0.009	V: 0.849 ± 0.002 B: 0.821 ± 0.003	V: 0.73 B: 0.66	0.825
C	3.989 ± 0.006	——	——	3.965
i (degrees)	86.34 ± 0.19	V: 87.8 B: 87.5	V: 77.2 B: 77.9	87.2
k	0.912	V: 0.829 ± 0.002 B: 0.841 ± 0.005	V: 0.81 B: 0.80	0.915
g	0.32	——	——	——
u_1	V: 0.799 ± .006 B: 0.864 ± .004	V: 0.6 B: 0.7	V: 0.60 B: 0.70	—— ——
u_2	V: -.125 ± .004 B: -.063 ± .003	—— ——	—— ——	—— ——
L_g	V: 0.550 ± .002 B: ——	V: 0.650 ± 0.008 B: 0.690 ± 0.007	V: 0.690 B: 0.679	—— ——
L_s	V: 0.450 ± .002 B: ——	V: 0.350 ± 0.008 B: 0.310 ± 0.007	V: 0.310 B: 0.321	—— ——
T_g	5660 K	——	5660 K	——
T_s	V: 6475 K	6336 K	——	——
a_g, b_g, r_g	.424 .393 .397	r_g: .417	r_g: .377(V) .393(B)	r_g: .402
a_s, b_s, r_s	.389 .357 .362	r_s: .345	r_s: .304(V) .316(B)	r_s: .368
A_1	0.5	V: 1.36 B: 1.20	——	——
A_2	0.5	V: 1.32 B: 1.47	——	——
F	1.01 ± 0.01	——	——	1.060
Y	V: 1.20 B: 1.49	V: 1.22 B: 1.42	—— ——	—— ——

most potential surface, $C > C_1$ (q). When the fillout is less than unity, the fillout is computed as the ratio of the innermost potential surface to the surface potential ($F = C_1/C$).

A system is *overcontact* when the fillout has a value between one and two ($1 \leq F \leq 2$). In this case the surface potential has a value which is greater than that of the outermost potential surface and less than that of the innermost potential surface, $C_1 \geq C \geq C_2$ (q). In this case on fillout is defined as:

$$F = (C_1 - C)/C_1 - C_2) + 1.$$

If the surface potential is less than that of the outermost potential surface (C_2) then the surface is no longer bounded and thus is not a region of interest in defining the surfaces of binary star systems. Table 5 shows that the two solutions having fillouts are very similar and just slightly overfill their inner Roche lobe.

Summary

Comparison of the solutions of the light curves of OO Aquilae shows some correlation, but there are also distinct differences in some of the parameters of the solutions. Part of the difference comes from the fact that the hot spot was included in only two of the solutions (present work and (28)), which also turn out to be the two most similar. Another reason for differences is that the solutions are based on different epoch light curves, some of which exhibit more asymmetry than others. The Binnendijk (1) data are approximately 20 years older than the data of Lafta and Grainger (10). The hot spot for the present solution covers 33.1% of the visible area of the binary system at elongation (phase 0.75). In addition, the hot spot is 30% intrinsically brigher than the surrounding area. This leads the authors to conclude that the secondary component is hotter than originally believed.

Acknowledgments

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PLANT TAXONOMY

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ABSTRACTS

Artificial Hybridization in *Solanum* sect. *Lasiocarpa*. CHARLES HEISER, Department of Biology, Indiana University, Bloomington, Indiana 47405.—*Solanum* sect. *Lasiocarpa* is a group of 13-14 species, largely confined to the Neotropics. Two species, *S. quitoense*, "Naranjilla," and *S. sessiliflorum*, "cocona," are cultivated. To those hybrids previously reported (Econ. Bot. 39: 4. 1985; Am. J. Bot. 74: 1045. 1987) may be added to the following: *S. hirtum* x *stramonifolium, S. lasiocarpum* var. *lasiocarpum* x *sessiliflorum* var. *georgicum, S. pseudolulo* x *hirtum, S. pseudolulo* x *stramonifolium, S. quitoense* x *hyporhodium*. Of these perhaps the most interesting are those involving *S. stramonifolium*, for this species appears to be rather distantly related to the others, yet its hybrids show some fertility and 12_{II} of chromosomes. In spite of numerous attempts it has yet been impossible to cross *S. pectinatum* with any of the other species. Three species in the section, *S. felinum* and *S. orientale* of Venezuela and *S. stagnale* of Brazil, have not been available for hybridization studies.

The fruit of the naranjilla gives a delicious juice. The plant is presently plagued by many diseases and once improved forms are available it will probably be more widely grown. The species shows very little variability and its improvement likely depends on interspecific hybridization.

A Full Text Data Storage and Retrieval System for Use in Plant Taxonomy. CLIFTON A. KELLER. Andrews University, Berrien Springs, Michigan 49104.—Wide availability of microcomputers and recent advancements in their technology has made feasible the storage of uncoded taxonomic data. A demonstration is given of a program able to provide ready access to the valuable and often hidden information in theses, monographs, floras, periodicals, glossaries, dictionaries and other frequently used literature.

Positional and Seasonal Variation in Pin Oak and Black Oak Leaf Morphology. KAREN E. LUDLAM AND RICHARD J. JENSEN, Department of Biology, Saint Mary's College, Notre Dame, Indiana 46556.—Eight trees, representing four individuals each of *Quercus palustris* Muenchh. and *Q. velutina* Lam., were examined for within- and among-tree variation in leaf morphology. All trees were sampled at two heights at each of the four cardinal compass directions and were sampled twice during the growing season. All variables were log-transformed and factorial analysis of variance was used to

assess patterns of variation in both the original measures and in size-corrected measures. Seasonal differences were evident in most variables, although size-correction lessened the number of significant effects. All variables illustrate significant among-tree differences while within-tree differences are primarily a function of season and position, compass direction generally illustrating negligible effects. Black oaks appear to illustrate greater overall variability as indicated by the number of significant higher-order interactions. Counts, angles, and symmetry were found to be less variable than linear measures, although complementary angles do not illustrate the same degree of variability.

The Bryophytes of Portland Arch Nature Preserve, Fountain City, Indiana. Bill N. McKnight, Indiana State Museum, Indianapolis, Indiana 46204 and Malcolm L. Sargent, School of Life Sciences, University of Illinois, Urbana, Illinois 61801.—The bryophytes (hornworts, liverworts, mosses, and peat moss) of the Preserve are discussed. Specific comments are offered on the total flora, geographic affinities, niche specificity, phenology, previous reports, rare species, frequency, state records, associated taxa, and peculiar morphological features. The physiography of this 253 acre west central Indiana Preserve also is discussed.

Phenetic Analysis of *Carex tetanica* complex (sect. *Paniceae*, Cyperaceae). Paul E. Rothrock, Department of Biology, Taylor University, Upland, Indiana 46989.—The *Carex tetanica* complex has been most commonly interpreted as consisting of three species: *Carex meadii* Dewey, *C. woodii* Dew., and *C. tetanica* Schkuhr. Alternatively, two or even only a single species with several varities have been recognized. In this study, herbarium samples from a broad geographic range were scored for 42 characters and analyzed phenetically using Euclidean distance, PCA, and traditional taximetric techniques. *C. woodii* proved to be easily separable from the other two taxa on the basis of culm coloration, beak angle, and achene shape, among other characteristics. On the other hand, in this preliminary analysis, *C. meadii* and *C. tetanica* did not form sharply circumscribable phena. Some characters, such as female spikelet L/W ratio and perigynia width, yielded high PCA vector scores and may be useful in recognition of subspecific categories.

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Additions to the Flora of Southern Indiana, III.

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Introduction

This paper is a continuation of a report published in 1986 on additions to the flora of southern Indiana (6). Southern Indiana is defined here as that area south of U.S. Highway 40 east of Indianapolis and U.S. Highway 36 west of Indianapolis. Information on current endangerment status in the state comes from Aldrich *et al.* (1). Natural region names come from Homoya *et al.* (7), and plant nomenclature primarily follows Crovello *et al.* (2). Most of the specimens discussed are to be deposited in the Deam Herbarium at Indiana University in Bloomington, Indiana (IND).

Species New To Indiana

Andropogon ternarius Michx. Harrison County. This species of beard grass was discovered growing in an old field adjacent to a natural sinkhole pond northwest of Laconia. The grass was common at the site. Although its occurrence may represent a vestige of the presettlement barrens that existed in the region, it is also possible that it is an example of a recent range expansion from the south. This is made in light of the fact that it colonizes old fields elsewhere in its range, such as other *Andropogon* species do here, and that it appears to have recently invaded nearby southern Illinois (Olson, pers. comm.). (August 26, 1987 *Homoya et al.* 87-08-26-49).

Species New To Southern Indiana

Aralia nudicaulis L. Ripley County. Several large patches of wild sarsaparilla were discovered growing in a mesic upland forest situated on the upper slope of a steep hillside in Versailles State Park. (May 16, 1986 *Homoya* 86-05-16-17).

Napaea dioica L. Ohio County. Formerly known to occur in Indiana only in the floodplain of the upper half of the Wabash River, this species was recently discovered on the south side of the valley where Laughery Creek flows into the Ohio River. Numerous individuals of this state-threatened plant were observed growing along roadsides and in small tributaries of Laughery Creek. (July 17, 1987 *Homoya and Martin* 87-07-17-38).

Panicum addisonii Nash. (= *Dicanthelium ovale* (Ell.) Gould and Clark var. *addisonii* (Nash) Gould and Clark). Vigo County. This grass, most common on the eastern coastal plain, occurs in widely scattered localities in the midwest. In Indiana, it is restricted to dry, sandy knolls where barrens vegetation predominates. In southern Indiana it was collected at the Little Bluestem Nature Preserve. (August 27, 1986 *Homoya* 86-08-27-70).

Infrequently Collected Species In Southern Indiana

Aster linariifolius L. Deam (3) listed three counties in southern Indiana for this attractive aster, these being Floyd, Harrison, and Perry counties. I have encountered this aster at only two sites in southern Indiana, once at Elizabeth Hill in Harrison County (a Deam collecting site), and once near Rockhouse Hollow in the Hoosier National Forest in Perry County. At the latter site the few plants noted were growing on a rocky crest of a hill dominated by chestnut oak. It was not collected.

Carex seorsa Howe. Jefferson County. The first discovery in southern Indiana of this sedge was made in Jackson County in 1985 (6). Since that time, a second population has been found, this one occurring in a vernal pool of a flatwoods natural community (May 15, 1986 *Homoya and Aldrich* 86-05-15-15).

Cirsium carolinianum (Walt.) Fern. and Schub. Perry County. This species, previously considered extirpated in Indiana, was discovered growing in three separate populations in and around limestone barrens in the Hoosier National Forest. This native thistle, which blooms in the spring, was referred to by Deam (3) as *Cirsium virginianum* (L.) Michx. Current nomenclature restricts that name to a plant confined to the southeastern coastal plain (4). (May 29, 1987 *Homoya and Spingarn* 87-05-29-11).

Eleocharis wolfii Gray. Ripley County. Cited as occurring only in Jefferson County in the Flora of Indiana (3), this state-endangered spikerush has since been collected in Spencer, Posey, and Lake counties, and now in Ripley County. The Ripley County specimens were collected in a vernally wet depression in a flatwoods. (July 16, 1987 *Homoya* 87-07-16-36).

Gentiana alba Muhl. Perry County. Three separate populations of this state-endangered gentian were found growing in a series of natural barrens in the Mogan Ridge area of the Hoosier National Forest. (September 10, 1987 *Homoya and Spingarn* 87-09-10-61). I recently observed, but did not collect, this species in Ripley and Franklin counties as well.

Glyceria acutiflora Torr. Owen County. Three collections of this grass, all made before 1936, were all that were known prior to the Owen County discovery. The populations of the historic collections are now apparently extirpated, leaving the Owen County population as the only extant one in Indiana. The new population was discovered growing in a small sinkhole pond situated high on a forested slope. The pond, dominated by buttonbush (*Cephalanthus occidentalis* L.), also has a large population of the rare log sedge, *Carex decomposita* Muhl. In Indiana, the *Carex* and the *Glyceria* are known from sinkhole ponds more than in any other habitat. This same phenomenon is true for these species in Missouri as well (8). (June 12, 1987 *Homoya and Brothers* 87-06-12-30).

Hypericum denticulatum Walt. Perry County. Previous collections of this species in Indiana were made in Spencer, Daviess, and Posey counties. The Daviess County and Posey County sites have been destroyed, thus leaving extant populations only in Spencer and Perry counties. The Perry County population occurs in a dry, old field dominated by little bluestem (*Andropogon scoparius* Michx.) Other rare species occurring in this old field include *Scleria pauciflora* Muhl. ex Willd. and *Spiranthes tuberosa* Raf. (August 7, 1987 *Homoya and Spingarn* 87-08-07-42).

Lechea villosa Ell. Harrison County. Reported by Deam (3) as occurring in three southern Indiana counties, I had never encountered this species until discovering it in a natural barrens remnant south of the town of Central Barren. (August 26, 1987 *Homoya, Abrell, Hutchinson, and Stritch* 87-08-26-48).

Ophioglossum engelmannii Prantl. Perry County. As more limestone glades are discovered in southern Indiana, so are occurrences of the limestone adder's tongue fern. To date, seven sites are known to harbor this state-threatened species in Indiana. The newest populations are located in limestone glade in the Hoosier National Forest west of Derby. (May 29, 1987 *Homoya and Spingarn* 87-05-29-10).

Penstemon deamii Pennell. Clark County. According to the authority on *Penstemon deamii* and its allies (Koelling, pers. comm.), *Penstemon deamii* is an Indiana endemic (apparently our only vascular plant endemic). Restricted to the Highland Rim Natural Region, collections of the species have come from Crawford, Harrison, Floyd and Clark counties, with the bulk from the latter. The specimens collected in Marion County that were determined to be *Penstemon deamii*, as cited

by Deam (3), apparently no longer exist. Given the species' restricted range and habitat, it is possible that they were incorrectly identified. The recent Clark County collections were made from roadsides and steep, eroding slopes in Clark State Forest. (May 29, 1986 *Homoya and Brothers* 86-05-29-26).

Poa wolfii Scribn. Spencer and Warrick counties. With the exception of an Owen County collection, all of the previous collections of this rare grass have come from northern Indiana. It was a surprise then to find it occurring rather commonly in the dry flatwoods of the Driftless Section of the Southwestern Lowlands Natural Region. This *Poa* species is one of the rarest of the genus in Indiana, with only four extant populations known. (May 28, 1987 *Homoya and Spingarn* 87-05-28-9).

Scutellaria saxatilis Riddell. Crawford County. Once thought to be extirpated from Indiana, this rock-loving skullcap was rediscovered at a Deam collecting site near the old Carnes Mill on the Little Blue River. Hundreds of plants were found growing on the tops of large, detached sandstone boulders. This location is considerably disjunct from the main area of the species' range, that being from eastern Ohio to eastern Tennessee and eastward. (July 11, 1986 *Homoya* 86-07-11-61).

Trifolium relexum L. Posey County. Several plants of this native clover were found growing in the skid trails of a recently timbered flatwoods in SW Posey County. Formerly thought to be extirpated, this is the only extant site in the state. (July 13, 1986 *Aldrich and Spingarn* 71386).

Trifolium stoloniferum Muhl. ex A. Eaton. Ohio County. Two separate populations of this federally-listed endangered species [Federal Register 52(108): 21478. 5 June 1987] were discovered growing in and around small rocky streams that flow directly into the Ohio River. Two juvenile individuals, each appearing to be *Trifolium stoloniferum*, were discovered in Switzerland and Ripley counties. Additional details are planned to be published elsewhere. (June 16, 1987 *Aldrich, Homoya, and Jacquart* 61687, and *Homoya, Aldrich and Jacquart* 87-06-16-31).

Veronica grandiflora Gaertn. Union County. This species, referred to commonly as the brook-pimpernel, is one of springs and spring-fed streams. Several plants were found in this habitat at Whitewater State Park. Synonymous with *Veronica grandifera* Pennell, this species was recently added to the list of state rare species. (July 2, 1986 *Homoya* 86-07-02-52).

Woodwardia areolata (L.) Moore. Perry County. In the first report of this fern for the state (5). I commented about the phenomenon of range expansion that the species appears to be undergoing at the northern edge of its primary range. Consistent with this phenomenon is the discovery of another Indiana location, this one on a roadbank in the Mogan Ridge area of the Hoosier National Forest. Interestingly, the fern was growing in the shade of another rare species in Indiana, the sourwood (*Oxydendrum arboreum* (L.) DC.) (August 7, 1987 *Homoya and Spingarn* 87-08-07-44).

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Indiana Plant Distribution Records 10/21/87

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Distribution records are listed for the southern counties of Clark, Floyd, Harrison and Washington. Genera are listed in the order of their appearance in Deam's *Flora of Indiana* (3); species within each genus are in alphabetical order followed by the county in which they were collected. Nomenclature is in accord with that used in Gray's *Manual of Botany*, 8th ed., 1950 (4), unless modified as noted (2,5,6). State endangered (e), threatened (t), or rare (r) plants are also noted (1).

Specimens were collected through the Indiana University Southeast herbarium (JEF), and through botany classes at IUS by the following: Sheila Ancona, Karen Boha, Ann Chambers, Marjo Chanley, John Coffey, Jerry Conrad, Denise Edgcomb, Belinda Eve, Raymie Gill, Jeff Harmon, Sandy Hensley, Charles Henson, Juliet Heun, Mike Howard, Deborah Kepley, Sheila Klein, Janice Loftin, Richard Maxwell, Francis Roberts, Lori Saulman, Richard Schoen, Cheryl Sommerville, Betty Staten, Larry Stevens, Jim Stephenson, Jeff Stoner, Doug Trusner, Kent Watson, and Lou Ann Windell.

Voucher specimens for all new records are in the Indiana University herbarium at Bloomington (IND). The author thanks Lewis Johnson, the curator, for his valuable help with the determinations. Doug Trusner provided valuable field assistance, and Stacey Stratton helped in the IUS herbarium.

Many of the Floyd county records were collected in the Brock-Sampson Nature Preserve with a permit from John Bacone, Director, Indiana Nature Preserves. The permit is greatly appreciated. Clark County records were collected in the Indiana Army Ammunition plant, Charlestown, with permission from the U.S. Army and cooperation of ICI, Americas, Inc., operating contractor, and this support is also greatly appreciated.

Taxonomic Entities

Ophioglossum engelmannii (1e), Clark. *Lycopodium digitatum* (2) (= *L. complanatum* L. var. *flabelliforme* Fern. (4,5); = *L. flabelliforme* (Fern.) Blanch. (6)), Clark, Harrison. *Botrychium virginianum*, Floyd. *Asplenium platyneuron*, Floyd. *Cystopteris fragilis* var. *fragilis*, Floyd. *Cystopteris fragilis* var. *protrusa* (= *C. protrusa* (Weatherby) Blasdell (2)), Floyd. *Pellaea atropurpurea*, Clark.

Typha latifolia, Floyd. *Diarrhena americana*, Clark. *Hystrix patula* (= *Elymus hystrix* L. (6)), Floyd. *Carex eburnea* (1t), Clark. *Eleocharis elliptica* var. *compressa* (6), Clark. *Luzula echinida* (= *L. multiflora* (Retz.) Lej. var. *echinida* (Small) Mohl. (6)), Floyd. *Smilax bona-nox*, Floyd. *Smilax hispida* (5,6), Floyd. *Trillium grandiflorum*, Floyd. *Trillium nivale*, Washington. *Dioscorea quaternata*, Floyd. *Spiranthes ovalis* (1t), Clark.

Juglans nigra, Harrison. *Ostryra virginiana*, Clark. *Chenopodium album*, Floyd. *Dianthus armenia*, Floyd. *Nelumbo lutea*, Floyd. *Actaea pachypoda*, Floyd. *Alliaria petiolata* (= *A. officinalis* (4,5,6)), Clark, Harrison. *Draba verna*, Floyd. *Agrimonia parviflora*, Floyd. *Amelanchier arborea*, Floyd. *Crataegus intricata* (1r), Floyd. *Duchesnea indica*, Floyd. *Rubus occidentalis*, Floyd. *Rosa multiflora*, Floyd.

Amorpha fruticosa, Harrison. *Vicia villosa*, Floyd. *Oxalis corniculata*, Clark. *Ailanthus altissima*, Floyd. *Euonymus americanus*, Floyd. *Hypericum drummondii*,

Floyd. *Hypericum perforatum*, Floyd. *Opuntia humifusa* (= *O. compressa* (Salisb.) Macbr. (6)), Floyd.

Epilobium coloratum, Floyd. *Aralia racemosa*, Floyd. *Ligustrum vulgare*, Clark, Floyd. *Asclepias viridiflora*, Clark. *Hydrophyllum canadense*, Floyd. *Heliotropium tenellum* (1e,5,6), Clark. *Blephilia hirsuta*, Floyd. *Datura stramonium*, Floyd. *Nicandra physalodes*, Floyd. *Gerardia pururea*, Floyd. *Paulownia tomentosa*, Floyd. *Veronica polita*, Floyd. *Ruellia humilis*, Clark. *Cephalanthus occidentalis*, Floyd. *Galium circaezans*, Floyd.

Arctium minus, Floyd. *Bidens bipinnata*, Floyd. *Cacalia atriplicifolia*, Floyd. *Erigeron annuus*, Floyd. *Eupatorium fistulosum*, Floyd. *Eupatorium perfoliatum*, Floyd. *Galinosoga ciliata*, Floyd. *Gnaphalium obtusifolium*, Floyd. *Hieracium pratense*, Floyd. *Lactuca floridana*, Floyd. *Solidago juncea*, Floyd. *Vernonia fasciculata*, Floyd.

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Some Recent Indiana Plant Distribution Records

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Introduction

From 1983 to 1986 various Indiana counties have been inventoried for potential natural areas. This has provided the opportunity to collect vascular plants for further identification. As a result of this work 82 new county records were collected from 28 counties. Included in these records were 14 plants considered endangered, threatend or rare in Indiana (1). Plant distribution records published in the *Proceedings of the Indiana Academy of Science* were checked by Lewis Johnson in order to determine new county records published since Deam (2).

Nomenclature follows Deam, except for *Andropogon gerardi* and *Chorispora tenella* which follow Swink (5), *Carex socialis* which follows Mohlenbrock and Schwegan (3) and *Carex atlantica* subsp. *atlantica* which follows Reznieck and Ball (4). Species are listed by the order in which they appear in Deam. Most specimens discussed are deposited in the Deam Herbarium at Indiana University in Bloomington, Indiana with some duplicates deposited in the herbarium at The Morton Arboretum, Lisle, Illinois.

New County Records—Endangered, Threatened or Rare Species

Endangered Species—*Lycopodium tristachyum* Pursh.—LaPorte County, *Carex atlantica* subsp. *atlantica* L. H. Bailey—Clay County, *Talinum rugospermum* Holzinger—Jasper County, *Aralia hispida* Vent.—Jasper County, and *Androsace occidentalis* Pursh.—Jasper County.

Threatened Species—*Lycopodium clavatum* L.—Pulaski County, *Lycopodium obscurum* L.—Pulaski County, *Carex decomposita* Muhl.—Owen County, *Carex seorsa* E. C. Howe—LaPorte County, *Carex socialis* Mohlenbrock and Schwegman—Warrick County, and *Waldsteinia fragarioides* (Michx.) Tratt—Greene County.

Rare species—*Carex alata* Torr.—Noble County, *Pyrola elliptica* Nutt.—Jasper County, and *Pyrola rotundifolia* L. var. *americana* (Sweet) Fern.—LaPorte County.

Other County Records

Ophioglossum vulgatum L.—Clay County, *Dryopteris cristata* (L.) A. Gray—Clay, Owen Counties, *Lycopodium flabelliforme* (Fern.) Blanchard.—Clay, Green, Owen Counties, *Lycopodium lucidulum* Michx.—Green, Owen Counties, *Andropogon gerardi* Vitamin—Jay County, *Zizania aquatica* L.—Pulaski County, *Carex convoluta* Mack.—Marshall County, *Carex davisii* Schwein. and Torr.—Vermillion County, *Carex emmonsii* Dewey—LaPorte County, *Carex grayii* Carey—Boone County, *Carex hyalinolepis* Steud.—Hancock County, *Carex laevivaginata* (Kukenth) Mack—Clay County, *Carex lasiocarpa* Ehrh.—Noble County, *Carex prasina* Wahl.—Clay County, *Carex meadii* Dewey—Jasper County, *Carex rostrata* Stokes—Starke County, *Carex swanii* (Fern.) Mack—Clay County, *Carex stipata* Muhl.—Hancock, Starke Counties, *Carex stipata* var. *maxima* Chapm.—Hancock County, *Carex tribuloides* Wahl.—Hancock County, *Scirpus polyphyllus* Vahl.—Greene County, *Juncus acuminatus* Michx.—Pulaski County, *Juncus biflorus* Ell.—Greene County, *Juncus balticus* Willd. var. *littoralis* Englem.—Lagrange County, *Juncus macer* S. F. Gray—Greene County,

Juncus marginatus Rostk.—Greene County, *Erythronium albidum* Nutt.—Clinton County, *Trillium gleasoni* Fernald forma *walpolei* (Farw.) Deam—Jackson County, *Spiranthes cernua* (L.) Richard—Greene County, *Quercus stellata* Wang—Vigo County, *Ranunculus flabellaris* Raf.—Sullivan County, *Menispermum canadense* L.—Sullivan County, *Liriodendron tulipifera* L.—Starke County, *Chorispora tenella* (Willd.) D.C.—Marion County, *Draba verna* L.—Tippecanoe County, *Sedum ternatum* Michx.—Jay County, *Filipendula rubra* (Hill) Robinson—Owen, Vigo Counties, *Potentilla fruticosa* L.—Porter County, *Euphorbia esula* L.—Boone County, *Rhus vernix* L.—Owen County, *Ilex verticillata* (L.) Gray—Clay, Henry, Owen Counties, *Rhamnus cathartica* L.—Newton, Starke Counties, *Rhamnus frangula* L.—Jasper, Jay Counties, *Ascyrum hypericoides* L. var. *multicaule* (Michx.) Fern.—Greene, Owen Counties, *Viola pedata* L.—Jay County, *Opuntia humifusa* Raf.—Marshall, White Counties, *Gaultheria procumbens* L.—Fulton County, *Monotropa hypopithys* L. var. *rubra* (Torr.) Farw.—Starke County, *Lysimachia lanceolata* Walt.—Vermillion County, *Forestiera acuminata* (Michx.) Poir.—Sullivan County, *Gentiana andrewsii* Griesb.—Owen County, *utricularia gibba* L.—Jasper County, *Mitchella repens* L.—Vigo County, *Krigia virginica* (L.)—Vigo County and *Matricaria matricarioides* (Less.) Porter—Sullivan County.

Acknowledgments

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ABSTRACTS

Impact of Parental Conflict and Father Closeness on the Development of Children's Heterosexual Relationships. Stephanie Lawrence, 2875 South 6th Place #2, Terre Haute, Indiana 47802. Michael R. Stevenson, Department of Psychological Science, Ball State University, Muncie, Indiana 47306.—The primary goal of this study was to assess the quality of participants' intimate heterosexual relationships as determined by their relationships with their fathers and the amount of conflict between parents as observed by the children. Two hundred two single, white students between the ages of 18 and 22 years participated. One hundred two were male and 100 were female. Participants answered questionnaires which consisted of a demographics section, seven items which reported conflict between parents, six measures which assessed their current heterosexual relationships, and two measures which assessed past and present relationships with fathers. Respondents who reported closeness to their fathers also reported satisfaction with their dating activities. Females were more likely to report loving and liking their partners, and also reported more intimacy with their partners. Both males and females were more likely to report more liking and loving of their partners if they also reported minimal parental conflict.

Personality Changes of American Teenage Participants in a Japanese Youth Exchange. Michael H. Stitsworth, Department of 4-H Youth, Purdue University, West Lafayette, Indiana 47907.—The purpose of this study was to determine whether changes in personality occur in teenagers during one-month homestays in Japan. The sample consisted of 154 exchangees and 112 control group members who did not travel abroad. The California Psychological Inventory was administered to both groups prior to the exchange, again at its conclusion, and a third time four months later. Antecedent information was collected using a pre-exchange questionnaire. Analysis of co-variances was used to determine if the pretest and posttest scores for the two groups were significantly different. Nonparametric tests were used to determine if certain overseas group antecedent subpopulations changed differently. The overseas group increased in flexibility and independence and became less conventional as compared to the control group. Exchangees who were the first member of their family to travel abroad and those who personally paid a high percentage of their trip expenses changed the most. Travelers who had studied a foreign language for one or two semesters experienced no significant changes; those with no previous language study and those who had studied a language for three or four semesters changed significantly.

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Attributions for Examination Success or Failure in an Undergraduate Sociology Course

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Introduction

Attribution theorists and researchers are interested in individuals' perceptions of "causes" of events or outcomes. Such perceived causes are termed "attributions." Attribution work has been summarized and used by Heider (3) and Weiner (7). Attributions have been grouped into several dimensions. The major dimensions are: stable or unstable (e.g., ability or luck), internal or external (e.g., effort or task difficulty), and controllability or uncontrollability (Weiner, (6)). The researcher may then want to know how the individual develops from these attributions, explanations for life occurrences. Attributions for successes and failures have been found to be related to self-esteem (1) and school performance (4). In some cases gender differences have been reported (2). It has also been suggested that these sex differences may depend upon the sex typing of the specific task.

Whitley, McHugh, and Frieze, in 1986 (8), reported a metaanalysis in which they examined all the available literature on sex differences in attributions. Only two consistent sex differences were found: 1) men made more attributions to ability both when they succeeded and when they failed, and 2) men made less use of luck both in success and failure.

One problem with these conclusions is that this analysis did not take into account the specific task involved in each study. Tasks may be categorized according to sex type. This refers to how expected or acceptable it is for a male or female to do, or achieve in that task. Another task distinction is whether the task is real life or artificial.

One suggestion that this might make a difference comes from a study done by Nicholls (5). This study involved the collection of attributions first for a practice test, and then for a true test. Findings showed women more likely to attribute failure to lack of ability in the practice test condition, but there were no significant sex differences in the ability attributions after the true test.

This paper is concerned with success and failure among college students in relation to "before" and "after" exam attributions. That is, it looks at how expectations affect attributions for successful or unsuccessful performance on an exam. For purposes of this study, success is defined as whether or not the students' performance matched their expectations. Students could perform higher than, equal to, or lower than they expected. In keeping with prior research, sex differences in attribution is also of interest. That is, we asked whether or not men and women differ in their patterns of attributions based on their expected performance.

Methods

Subjects and Design

Data for this study were collected during the Spring semester of 1985, using the population of students enrolled in one section of Introductory Sociology at Purdue University. Before the first exam the subjects were given a four page questionnaire that contained questions about the following: general background information (i.e., age, race, grade point average, school classification, etc.); self-esteem as a student; expectations about grades in this particular course; probable attributions for hypothetical test results that did not match expectations; and perceived images of subjects as students held by significant others. After the first exam a second questionnaire was distributed to obtain the reported exam scores and the explanations for those scores. This sequence of before and after questionnaires was repeated for the subsequent two exams, with the final questionnaire to be mailed to the researchers when the subjects obtained their final exam scores. Because of the low return rate on the final questionnaire, only the first five questionnaires were analyzed. Complete sets of five questionnaires were obtained from 134 students, representing 29.7% of the enrolled class. The subjects were mainly white (97.5%), and three-fourths were freshmen or sophomores. About 55% were females and 45% were males.

Measures

For the purposes of this paper, only a portion of the questionnaire was analyzed. The following information was used:

A summated score was developed for the following series of 10 questions to obtain a self-esteem score. With the four point scale, 40 was the highest self-esteem score possible.

> Now we would like to ask you some questions about how you feel about yourself as a *student*. For each of the following statements, please indicate how strongly you agree or disagree.
>
> AS A STUDENT SA A D SD
>
> a) At times I think I am no good at all . . . .
> b) I certainly feel useless at times. . . .
> c) On the whole, I am satisfied with myself. . . .
> d) I wish I could have more respect for myself. . . .
> e) I take a positive attitude toward myself. . . .
> f) I feel I do not have much to be proud of. . . .
> g) I am able to do things as well as most other people. . . .
> h) All in all, I am inclined to feel that I am a failure. . . .
> i) I feel that I have a number of good qualities. . . .
> j) I feel that I'm a person who's worth something, at least equal to others . . . .
>
> Assuming a total of 100 points on the exam, how many points do you expect to get? ____ points out 100
>
> From the "after" questionnaire the following information was used:
>
> Was your grade on this exam:
> ____ Higher than expected?
> ____ About what was expected?
> How many points? ____
>
> To what extent was the grade you received due to your academic ability?
>
> weakly 1 2 3 4 5 6 7 strongly

How much personal control did you have over the grade you received?
very little 1 2 3 4 5 6 7 a great deal
To what extent do you feel that the grade you received was really based on chance factors such as good or bad luck?
very little 1 2 3 4 5 6 7 a great deal
To what extent was the grade you received due to your own personal efforts, such as studying hard, reading all the material, coming to class, etc.?
very little 1 2 3 4 5 6 7 a great deal
To what extent was the grade you received due to how difficult you thought the exam was going to be?
very little 1 2 3 4 5 6 7 a great deal

Results

Mean self-esteem scores were figured for males and females at all three time periods. There were no differences in the means for the sexes, with mean esteem scores for the various times and sexes ranging from 30.3 to 32.1. Means were then figured for each attribution category (ability, personal control, luck, effort, and task difficulty) by success level as well as by sex. As the responses were based on a seven point Likert scale, a value of four is neutral. (See Tables 1 and 2.) Overall, luck was not viewed as a major component of outcomes.

For purposes of analysis, the subjects were divided into success categories according to whether their grades were "higher than expected," "expected," or "lower than expected." In the "grade higher than expected" and "grade expected" categories,

TABLE 1. Means and Standard Deviations of Attributions by Sex and Success Level.

Time 1

Grade higher than expected

	Male			Female		
	Mean	S.D.	N = 8	Mean	S.D.	N = 8
Ability	5.4	.74		5.6	1.06	
Pers. Cont.	5.4	.92		5.6	1.06	
Luck	2.9	1.46		3.3	1.91	
Effort	5.3	1.58		6.3	1.04	
Task	4.4	1.92		4.6	1.60	

Grade expected

	Male			Female		
	Mean	S.D.	N = 33	Mean	S.D.	N = 36
Ability	5.2	.91		5.5	1.18	
Pers. Cont.	5.5	1.09		5.5	1.13	
Luck	2.8	1.56		2.9	1.33	
Effort	5.9	.93		6.1	.97	
Task	4.0	1.44		4.4	1.52	

Grade lower than expected

	Male			Female		
	Mean	S.D.	N = 20	Mean	S.D.	N = 28
Ability	4.1	1.50		3.8	1.39	
Pers. Cont.	5.1	1.21		4.8	1.55	
Luck	3.1	1.36		3.0	1.67	
Effort	5.3	1.38		5.0	1.59	
Task	3.7	1.48		4.4	1.45	

TABLE 2. Means and Standard Deviations of Attributions by Sex and Success Level.

Time 2

Grade higher than expected

	Male			Female		
	Mean	S.D.	N = 8	Mean	S.D.	N = 9
Ability	5.4	1.41		5.9	.78	
P.C.	6.0	.00		6.2	.67	
Luck	3.1	.99		2.8	1.20	
Effort	5.9	.35		6.4	.53*	
Task	4.8	1.17		4.4	.88	

Grade expected

	Male			Female		
	Mean	S.D.	N = 27	Mean	S.D.	N = 32
Ability	4.8	1.24		5.3	1.05	
P.C.	5.6	1.01		5.8	.87	
Luck	2.7	1.30		2.8	1.25	
Effort	5.7	1.14		5.9	.84	
Task	4.0	1.32		4.3	1.36	

Grade lower than expected

	Male			Female		
	Mean	S.D.	N = 22	Mean	S.D.	N = 28
Ability	4.0	1.32		4.0	1.63	
P.C.	4.9	1.58		4.2	1.64	
Luck	3.6	1.84		3.9	1.56	
Effort	4.8	1.44		4.4	1.97	
Task	4.0	1.60		4.4	1.22	

for both sexes, the attributions with the consistently highest mean values were ability, personal control, and effort. For both Time 1 and Time 2 attributions, in the "grade lower than expected" category, the highest values were still given to effort and personal control, implying that there was a lack of effort on the part of the respondents and that the respondents felt that they had personal control over the outcome.

One way to test for sex differences is to do t-tests. When this was done, the only significant sex difference occurred in the strength of Time 2 attributions to effort in the "grade higher than expected" category. Females made significantly higher attributions to effort ($M = 6.4$) than did males ($M = 5.9$) ($t = 2.58$ and $p = .02$). However, with 30 comparisons this is likely to be a chance finding.

An analysis of variance by sex and success level for each attribution category showed six significant main effects, two for Time 1 and four for Time 2. In each of these six cases, the attribution difference was explained not by the sex but by the success level of the respondent.

At Time 1, attributions to ability and effort differed by success level. At Time 2, these factors as well as personal control and luck differed by success level. Ability had slightly weaker attributions in the "lower than expected" results category than in the "higher than expected" or the "expected" categories. This means that the subject who performed at an expected or higher level said that the cause was an inherent ability but those who performed poorly did not say that the results were caused by a lack of ability. Rather, those who performed at a level "lower than expected" said that the results were caused by a lack of effort. At Time 2 only, the lowest rating for personal control was in the "lower than expected" category. Instead, respondents were more likely to use luck as an explanation.

Discussion

The findings of this study are consistent with those of other researchers (8), with the exception of a lack of sex differences. There were no differences between the sexes in the self-esteem levels across the time periods. In this study, women and men reported comparable levels of feelings of worth as students. Despite past suggestions of some sex differences in attribution patterns, in this study, using attributions concerning a real life event, the determining factor for attribution pattern was the level of success achieved by the subject.

The discrepancy between earlier findings of sex differences and the lack of sex differences in this study may be a result of any or all of three factors. That is, one possiblity is that the women in college are now making attributions more similar to those traditionally made by men. Another possibility is that an Introductory Sociology class has no specific sex typing, and therefore neither gender is "supposed to" perform in a particular manner. Finally, the use of a real life situation may, as in the Nicholls study (5), lessen sex differences.

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Informational Needs of Single Parents

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Introduction

The significance of research on single-parent families is evidenced by the growing number of these families. In 1982, more than 8 million families in the United States were headed by a single parent (13). Glick (6) has estimated that by 1990 fifty percent of children will reside in a one-parent home for some period of time before they reach the age of 18 years.

The impetus for much of the research on single-parent families has been a theoretical interest in the family's influence on child development. However, research on single-parent families has also had an impact on child custody proceedings (9, 15) on therapy and counseling with single-parent families (2, 7), and on social policy toward single parents (1, 3, 4, 7). Further, such research may help to correct potentially erroneous and harmful assumptions that many hold about the effects of single-parenting (10, 15). It has been argued that these assumptions may be as harmful to single-parent children as any direct effects that parental divorce or deaths may cause (5, 3, 15).

Although interest in single parenting has been increasing steadily, single parents have rarely been consulted concerning their information needs. The relevant literature shows that single-parent fathers want to know how to be a nurturing parent (12) and about what constitutes "normal development" (11). Perhaps this is due to the pressures single fathers feel to be a nurturant parent (14).

Turner and Smith (16) have stressed the single parent's need for education regarding the selection of quality day-care service (16). Finally, Hughes and Durio (8) demonstrated that single parents have different information needs when compared to other types of families.

Few studies have addressed single parents' preferences in regard to the source of information. Hughes and Durio (8) showed that in the absence of an ex-spouse, single parents were more likely to consult no one about their child care concerns. Mendes (11) showed that single fathers found books to be a useless source of information. Orthner, Brown & Ferguson (12) recommended that classes on single parenthood be offered. However, they offer no data regarding single parents willingness to attend such a course.

The present research was designed to investigate the information needs of single parents. The results show what kinds of information single parents need and how they go about getting this information.

Method

Participants were members of Parents Without Partners (PWP) of Central Indiana. Twenty-six members (20 females, 6 males), aged 29-59 years (mean 40 years) participated in the study. Participation was voluntary and anonymous. Participants were asked to complete questionnaires that were distributed through the PWP newsletter and Chapter meetings. Permission to complete the study was granted by the regional president and board of directors of Central Indiana.

The survey questionnaires consisted of a demographic questionnaire and an information needs questionnaire. Demographic information included family type, ethnicity,

occupation, education, salary and support, gender, age, number and age of children and length of time spent as a single parent. Participants were also asked to rate the extent of their interest in obtaining more information in areas such as: Child development, child care, school behavior, the effects of single-parenting on children, the stress of being a single parent, handling family finances, financial planning and including children in discussions of family finances. Participants were then asked to indicate where they obtained information concerning child development, single-parenting and financial matters. Potential sources of information included: parents, ex-spouse, friends, clergy, doctors, teachers, newspapers, magazines, books, professional counselors and PWP meetings.

Results and Discussion

Frequency data for demographic characteristics, informational needs and information sources were tabulated. Participant's educational background ranged from completion of 7th grade through graduate school. Sixty-nine and three-tenths percent had attended college or were college graduates. Ninety-two percent were divorced and length of time as single parent ranged from 1-15 years (mean 5 years, mode 2 years). Ninety-six percent were engaged in paid employment with weekly take home pay ranging from $50-$500 (mean $280.00, mode $350.00). Fifty-eight percent were receiving child support or alimony.

TABLE 1. *Percentage of Single Parents Interested In Gaining More Information*

Child Development	Very Interested	Mildly Interested
Handling Discipline Problems	69%	23%
Involving Children in Family Decisions	62%	31%
Typical Child Development	23%	26%
Characteristics of Quality Day Care	19%	15%
Single-Parenting		
Effects of Remarriage on Children	77%	4%
How to Deal with Task Overload	73%	27%
Children's Interpersonal Relationships	62%	31%
Tell Children About Parent's Sexual & Dating Behavior	58%	27%
How to Deal with Stress	62%	35%
Maintaining Relationships with Former In-Laws	26%	30%
What to Tell About the Absence of a Parent	26%	5%
Effects of Moving on Children	30%	23%
Effects of Changes in Visitation & Custody	31%	39%
Financial		
Get Most from Local Social Services	46%	23%
Information on Financial Planning	46%	35%
Tell Children About Family Finances	31%	46%

Results indicated that single parents were interested in developmental issues concerning the handling of discipline problems, children's behavior at school and involving children in family decisions. Developmental information was acquired from magazines, friends, books, teachers and PWP meetings.

Single parents were extremely interested in information concerning the issues unique to single-parenting. These interests included how single-parenting effects the child's adjustment, sex-role development, and interpersonal relationships; what to tell children about the parent's dating and sexual behavior and the effects of remarriage on children. Single parents were also interested in the effects of their roles on themselves regarding issues of stress and dealing with task overload (too many responsibilities and too little time). Single-parenting information was acquired from friends, magazines, PWP meetings, newspapers and books.

Results show some interest in basic financial planning, the effective use of local social services and what to tell children about family finances. Financial information was acquired from friends, magazines and newspapers.

TABLE 2. *Percentage of Single Parents Who Acquire Information From Various Sources:*

Child Development
Magazines 73%
Friends 62%
Books 58%
Teachers 54%
Professional Counselors 23%

Financial
Friends 58%
Magazines 50%
Newspapers 42%
Professional Counselors 19%

Single-Parent
Friends 69%
Magazines 69%
PWP 54%
Books 34%
Professional Counselor 23%

When looking at what single parents are interested in, it might also be useful to discern what information they aren't as interested in receiving and what sources aren't used. Results indicated that single-parents weren't as interested in typical child development, the nutritional needs of children or characteristics of quality day care. This may be true in our sample because most of the participants children were beyond day care ages. The majority of the children were between the ages of 12 and 28 years.

Single parents were also less interested in information concerning what to tell the children about the absence of a parent, the effects of moving on children, the effects of changes in visitation and custody and information on maintaining relationships with former in-laws. This may also be a function of the older ages of the children and the number of years since the divorce. The mean number of years since divorce was 5, because of this the participants had already dealt with these issues. Single parents weren't as likely to seek information from parents, ex-spouse, clergy, and doctors.

Results also indicate that in these three areas, only 19-23% of the participants would seek this information from a professional counselor. However, 73% stated that it was very likely or likely that they would attend a course on single-parenting if one were available. This information is important to professional counselors and educators, who may attempt to provide needed information to single parents.

Conclusion

With the increasing numbers of single parents and children coming from single parent families, it becomes apparent that their unique informational requirements need to be met. It is also apparent that these needs might not be met efficiently by more traditional methods of counseling and education. Efficient and effective dispersal of the information could be achieved in part by:

1) Single-parenting classes at local high schools and universities, which also provide on-site child care.
2) Professional consultation and outreach programs to reach the single parent rather than requiring them to come in for traditional counseling.
3) Increasing the number of high quality articles addressing the needs of single parents, written by professionals but published in popular magazines and written at a level that the layperson can understand.

In summary, the informational needs of single parents and the sources of this information are important when looking at the potential impact this family system has not only on its members, but on the members of society in general. This points to the need for additional research in this area and for innovative methods of disseminating this information to the group who could really benefit from this knowledge: single parents and their families

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SCIENCE EDUCATION

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ABSTRACTS

The Purdue Atmospheric Science Education Project Summer Program. MICHELLE E. AKRIDGE, GERALD H. KROCKOVER, DAVID R. SMITH AND JOHN T. SNOW, Department of Earth and Atmospheric Sciences, Purdue University, West Lafayette, Indiana 47907.——In July 1987, 26 Indiana middle school/junior high school teachers participated in a four week atmospheric science program. The program was supported by a grant from the National Science Foundation. It was designed to improve the atmospheric science backgrounds of the participants and to assist them in applying the acquired knowledge into their classrooms. The program staff consisted of a combination of atmospheric scientists and science educators in order to provide a good mix of science and methods. The program format was a daily schedule of atmospheric science lecture and three hands-on laboratories: a field lab, an analysis lab, and a physical lab. Educational applications seminars were also conducted to provide the teachers with an opportunity to develop implementation plans for atmospheric science materials and equipment.

Some of the program laboratory exercises as well as some of the plans for classroom implementation will be presented.

1800's American Observatory and Laboratory Equipment. VINCENT A. DINOTO, JR., Department of Physics, Jefferson Community College Southwest, Louisville, Kentucky 40272.——The physical science equipment of the early United States college laboratories and observatories will be discussed. The equipment of most of the early American laboratories was manufactured in Europe through the early 1800s. By the middle of the 1800s skilled craftsment began to construct much of the equipment for the college laboratories, while some of the major institutions of higher learning still purchased European equipment. In this paper the equipment manufactured by American craftsmen will be discussed with particular interest given to the Clarke and the Pope Companies.

The use of this historical equipment in the teaching of the physical sciences will also be explored.

Environmental Archaeology and Ethnobiology. GARY E. DOLPH, Indiana University at Kokomo, Komomo, Indiana 46902.——Almost unnoticed, two new topics have been slowly emerging in advanced placement and college level introductory botany and biology textbooks—the origin of agriculture and the form and function of domesticated plants and animals. To obtain background information on these topics, a wide variety of fine reference books are available. In contrast, practical field experience is more difficult to obtain. Therefore, I would like to discuss a very pleasant and cost-effective method of obtaining this experience. I would recommend studying Southwestern Indian (Anasazi) efforts at demostication and agriculture by attending the Crow Canyon Archaeological Center near Cortez, Colorado. Two sites, the Duckfoot Site (860 A.D. to 880 A.D.) and the Sand Canyon Pueblo (1232 A.D.

to 1274 A.D.), are being excavated by the Crow Canyon staff. I recommend working at the Duckfoot or similar Pueblo I Site, because at this time, the Anasazi were actively attempting to expand their agricultural resource base in the face of climatic deterioration and increasing population size.

Pick a Pocket. Mary L. French, Linton-Stockton Elementary School, Linton, Indiana 47441 and Stanley S. Shimer, Science Teaching Center, Indiana State University, Terre Haute, Indiana 47809.——This will show an activity that will help emphasize science and also control students. The procedure works as a behavior modification for students. We will give you the rules and a diagram of how it works and how it benefits students and teachers.

Physics Curriculum Modification to Include Holography in High School Optics Programs. Uwe J. Hansen, Department of Physics, Indiana State University, Terre Haute, Indiana 47809.——During the past two years the Indiana Commission for Higher Education has supported the Indiana State University physics department with Title II funds to conduct a series of workshops designed to prepare teachers to include holography in the high school physics curriculum. Minimal equipment was provided to participating schools for program implementation. Successes and difficulties in curriculum design will be discussed along with basic holography principles and their implications in course modification.

Holography in the High School Classroom. Paul Mason, South Vigo High School, Terre Haute, Indiana 47802 and Uwe J. Hansen, Department of Physics, Indiana State University, Terre Haute, Indiana 47809.——Enrollments in high school science classes have shown some alarming trends during the last few years. One approach to remedy the situation is to include topics in the curriculum which convey to the students some of the excitement of contemporary science. This is difficult since much of the frontier of contemporary physics involves sophisticated equipment inaccessible at the high school level. Holography is an exception to this dilemma. With relatively modest means acceptable holograms can be produced by students in a high school laboratory. Minimal equipment will be discussed and examples of production of single and multiple beam reflection and transmission holograms will be given.

Laboratory in Engineering Physics: Elastic Behaviour of Beams. John Ostendorf Department of Physics and Engineering, Vincennes University, Vincennes, Indiana 47591 and Uwe J. Hansen, Department of Physics, Indiana State University, Terre Haute, Indiana 47809.——Laboratory modules to explore principles of applied statics were developed and tested in a classroom setting. The modules comprise the following topics: (1) Determination of the modulus of elasticity of bending structural members. (2) The mathematics of bending Beams. (3) The Cantilever Beam with Concentrated Load. (4) The Cantilever Beam with Distributed Load. These laboratory modules were developed for a second year engineering physics curriculum, however preliminary classroom tests and evaluations indicate possible adaptability to a general advanced applied physics laboratory in a regular physics curriculum.

Defining the Limitations of Computers in Science Education. John Richard Schrock, Division of Biological Sciences, Emporia State University, Emporia, Kansas 66801.——When routine word-processing, record-keeping and data management are separated from bona fide educational tasks, the predicted advantages of computers in education have failed to materialize. Developments in semantics and cognition science and the failure of computers to accomplish simple tasks in "commonsense" and recognition

indicate innate limitations to computer use in science education. While this boundary of limitations cannot be finely defined, the following functions appear to be well beyond the abilities of computers: provision of word "meaning", reward of insightful non-standard answers, provision of non-audio-visual stimuli, provision of test-truthfulness, provision of real consequences, prediction in open systems, prediction in chaotic systems, rare and deviant cases for expert systems. However, most of these functions are easily provided by standard laboratory and field techniques. No justification is found for replacing any safe and practical laboratory exercise or field trip. The dangers of abandoning reality for simulations is illustrated by a newly marketed computer simulation that is elegant but erroneous.

How to Utilize Rose Colored Glasses. STANLEY S. SHIMER, Science Teaching Center, Indiana State University, Terre Haute, Indiana 47809.——This presentation will show how to make Rose Colored Glasses. I will describe how this will be implemented into an elementary classroom to teach science. It will involve the student with first-hand experiences, collecting data and raising questions. This will allow the students to explore the effects of various colors on observation.

Pan Am Animal Sciences Exchange: Internationalizing Animal Sciences Education. MICHAEL H. STITSWORTH, Department of 4-H Youth, Purdue University, West Lafayette, Indiana 47907.——A $18,200 grant awarded to the Department of 4-H Youth at Purdue University by The National Association of the Partners of the Americas is providing an exchange of Indiana 4-H club members and 4-S club youths in Rio Grande do Sul, Brazil. The Pan Am Animal Sciences Exchange permitted sending ten 4-H members enrolled in animal sciences 4-H project areas to Rio Grande do Sul for 6 week homestays during the summer of 1987. Purdue University faculty assisted the hosting Brazilian 4-S organization in structuring appropriate thematic experiences. The 4-H members lived on typical Brazilian animal production farms and toured agricultural training facilities, processing operations, and exporting facilities. In the summer of 1988, eight Brazilian 4-S club members will live on Indiana farms for six weeks to learn about animal science practices, research, and technology in Indiana.

Four-H, a program of the Purdue University Cooperative Extension Service, is Indiana's largest non-formal educational program with a membership of 160,000 youth distributed among Indiana's 92 counties.

SOIL AND ATMOSPHERIC SCIENCES

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ABSTRACTS

A World Soils and Terrain Digital Database. M. F. Baumgardner, Purdue University, West Lafayette, Indiana 47907.—Throughout the world land resource managers are constrained in their decision-making because of a critical lack of information about their soils resources. The International Society of Soil Science is beginning a project to improve the mapping and monitoring of world soils and terrain resources and to develop an information system capable of delivery of accurate, useful, and timely information about soils and terrain resources to decision-makers and policy-makers. The major tasks of the project are 1) creation of a "universal" legend for a world soils and terrain survey at a scale of 1:1M; 2) definition of parameters for entry into the database; 3) selection and scheduling of land areas to be added sequentially to the database; 4) acquisition and input of all data essential for inclusion in the database; 5) implementation of updating and overlay capabilities; 6) development of capability to extract interpretive information from the database; and 7) transfer of the technology to the user community.

A Comparative Study of Economical Rain Gauges. Shawn B. Harley and John T. Snow, Department of Earth and Atmospheric Sciences, Purdue University, West Lafayette, Indiana 47907.—Schools, hobbyists, and farmers interested in measuring rainfall often use small, inexpensive rain gauges. During 1986-1987, the testing and evaluation of several models of inexpensive rain gauges was carried out at the Cherry Lane Meteorological Observational Facility on the campus of Purdue University in West Lafayette. The catch of the small orifice rain gauges was compared to the catch of a standard National Weather Service eight inch dipstick rain gauge.

The tested rain gauges will be described, and the advantages and disadvantages of the different models presented. The results of the comparison tests, and the appropriateness of using small orifice rain gauges will be discussed.

Contribution of Organic Fractions to Soil Spectral Reflectance. T.L. Henderson, M.F. Baumgardner and D.E. Scott. Department of Agronomy and USDA-ARS, Purdue University, West Lafayette, Indiana 47907.—In recent years, high-altitude remote sensing has emerged as extremely important technology for monitoring the earth's agricultural resources. A thorough understanding of factors influencing soil

spectral reflectance is essential if satellite-generated remotely sensed data are to be applied successfully to soil survey. While it is well known that increased soil organic matter content causes an overall decrease in soil reflectance, the effect of individual soil organic fractions on reflectance at specific wavelength bands has not been investigated. This study attempts to identify the contributions of the humic and fulvic acid fractions to soil reflectance. Organic matter from four Indiana soils is extracted and then fractionated, purified and freeze-dried. Reflectance is measured in 10 nm bandwidths over a wavelength range of 0.52-2.32 μm with an Exotech 20C spectroradiometer. The reflectance curve for each organic fraction will be compared to that of the unalterd soil sample. It is expected that specific diagnostic features may be found in the reflectance curves for humic and fulvic acid which may be useful for soil characterization using remotely sensed data. Preliminary results will be discussed.

The DNA PHETS Dust Devil Census. Thomas M. McClelland and John T. Snow, Department of Earth and Atmospheric Sciences, Purdue University, West Lafayette, Indiana, 47907.—In June, 1985 a large dust devil damaged a critical piece of instrumentation during an experiment funded by the Defense Nuclear Agency at White Sands Missile Range (WSMR), New Mexico. To help prevent such accidents in the future, a dust devil study was conducted in the northern part of WSMR from mid-May through August, 1986 and again through April and May, 1987. The central part of the study was a census to determine the spatial and temporal distributions of dust devils in the area. Along with the dust devil occurrence data taken by observers, there are data available from a 10-meter meteorological tower located near the center of activity. Combining and analyzing these data sets have aided in determining the meteorological conditions favorable for dust devil formation. An overview of the census, some of the results, and some speculations on dust devil behavior will be presented.

Some Astrophysical Aspects of Drought. Gayther L. Plummer, Office of the State Climatologist. University of Georgia. Athens, Georgia.—Dry-weather patterns, often associated with 22-year sunspot cycles, recently with 18.6-yr lunar cycles, and with the planets historically, have frustrated forecasters because recurrences varied imprecisely. Relations to remote astrophysical factors are controversial, but such studies globally have been revived recently. Rainfall especially during April is distinctly patternistic; perhaps cyclic in both Indiana and Georgia. Dry-weather and droughts are related clearly to heliocentric positions and to geocentric distances of the planet Jupiter. Cause-effects are believed to be astromagnetic; that is, magnetic properties within the solar system seem to respond to the dynamo properties of this planet as well as some others. Also, cycles may vary in response, synergistically sometimes and antagonistically otherwise, to astromagnetic interferences. Future dry-weather periods are expected according to the patterns and cycles that appeared; that is, moderate droughts about 1997-1999 and extreme ones about 2010-2015.

Results of a Mass Balloon Launch over Indiana. John T. Snow, Mark D. Conner, Michelle A. Akridge, Thomas M. McClelland and Shawn B. Harley, Department of Earth and Atmospheric Sciences, Purdue University, West Lafayette, Indiana 47907.—On the morning of Saturday 18 July 1987 ninety-eight sets of small balloons, each with a return post card, were launched from the campus of Purdue University, West Lafayette, Indiana. This mass launch was carried out as part of the Purdue University Atmospheric Science Education Project, a summer program for middle school science teachers. As of the time of submission of this abstract, 11 post cards had been recovered, all from the general vicinity of Rochester, Indiana. This paper

describes the launch operations, the collateral experiments conducted to determine the properties of the balloon set, and the results obtained. Particular attention is given to relating the locations from which post cards were returned to likely balloon performance and a plausible trajectory model based on the upper winds observed at Salem, Illinois at 1200 GMT (7:00 AM EST) 18 July 1987. It appears likely that the balloons remained aloft about 3 to 4 hours, reached an altitude of around 18,000 feet, and descended due to diffusion of helium through the latex.

Use of Remote Sensing to Study the Soils of Hungary. A. Szilagyi and M. Baumgardner, Purdue University, West Lafayette, Indiana 47907.—Seventy-three percent of Hungary's 90,030 km^2 is cultivated. The soils of Hungary have been mapped at a scale of 1:10,000 for use in crop management, soil conservation, and land reclamation. Currently three soils research projects are being conducted by the Remote Sensing Centre established in 1981. The objective of one project is to develop an effective methodology of photointerpretation for large scale soil mapping. The objective of a second project is to prepare a manual of photointerpetation for soil and water conservation engineering, particularly in waterlogged areas. The third project involves the comparison of multispectral and multitemporal Landsat thematic mapper and SPOT data for soil and crop mapping in a test area of the Hungarian Plain. Preliminary results indicate that many anomalies related to crop conditions, waterlogging, alkalization and soil erosion can be delineated by computer-implemented spectral analysis of high resolution data.

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Interactions of Weather, Soils and Management on Corn Yields: A Case Study in Wabash County, Indiana—1983

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ABSTRACT

Interactions of Weather, Soils and Management on Corn Yields: A Case Study in Wabash County, Indiana—1983. It is often difficult to interpret the results of plot level research conducted on farm fields over areas and years. Analysis of data from a study of the effect of tillage system on corn (*Zea mays* L.) yields obtained by Wabash County farmers in 1983 initially suggested that the mean yields obtained differed significantly by tillage system. Precipitation in 1983 in Wabash County was highly variable, however, and much of the variation among the yields obtained from different farms and tillage systems appeared to follow patterns of moisture stress, an interaction between weather and soil conditions.

To evaluate the effect of moisture stress on corn for the different tillage treatments, soil and weather variables were combined to calculate the daily ratio of actual to potential evapotranspiration, ET/PET, using SIMBAL, a soil water balance simulation program which considers the effects of soil and drainage conditions. The daily ET/PET calculations were summed from 40 days before to 50 days after silk for each plot and included as a single variable, ΣET/PET, along with management variables in the statistical analysis. Tillage treatment differences were no longer highly significant in explaining mean yields after appropriately accounting for soil and precipitation differences among plots, although a no-till*stress interaction indicated that the effect of increasing moisture stress on yield changed with tillage system. While these results were obtained using only data from a single year, they indicate that weather and soil information has to be considered explicitly in the interpretation of farm level experiments.

Interactions of Weather, Soils and Management on Corn Yields: A Case Study in Wabash County, Indiana—1983

It is often difficult to extend the results of plot level research to production agriculture even though the research may have been conducted in different sites and over a period of years. To present mean research results from plot experiments without explicitly considering weather, soils, and management interactions under which the

research was conducted may lead to flawed extension advisories and farmers to make erroneous management decisions.

Although the adoption of soil conservation tillage systems has been increasing, there is reluctance on the part of many farmers to make changes which may reduce crop yields. There is little doubt that no-till and ridge till systems do conserve soil and water (5), but their effects on crop yields have been variable. Results from research on crop yield response to soil conservation tillage systems in Indiana have varied, ranging from generally no effect or slight yield increases over those with conventional tillage methods in southern areas to yield decreases in northern areas, primarily because soil temperatures may be lower under conservation tillage systems than with conventional tillage (6). Major factors have been soil drainage and prior crop, i.e., a system such as no-till does well on well drained soils even in heavy residue while on poorly drained soils no-till doesn't compete in heavy residues. Yields for other reduced systems such as chisel and ridge till are commonly assumed to not be affected to any great extent. To evaluate conservation tillage methods, the farmer needs to know that little, if any, effect on yields can be expected as he moves from conventional to reduced tillage systems.

In this study we examined the effect of tillage system on corn (*Zea mays* L.) yields obtained by Wabash County farmers during 1983. Although preliminary analysis of the data suggested that yields differed significantly by tillage system, most of the significant variation among corn yields seemed to follow moisture stress patterns, an interaction between weather and soil conditions. The objective of this study was to evaluate the effects of mositure stress* tillage interactions on yield response.

The weather in Wabash County during 1983 was cool and wet early in the growing season becoming hotter and much drier than normal in July and August. At the Wabash cooperative National Weather Service station, which has a 1951-1980 normal based on continuous observations, April received 129% of the normal rainfall, May 171%, and June 117%. July and August received 74%, and 41% of normal rainfall, and temperatures averaged 1.9 and 2.6°C above normal, respectively (13). Wabash County corn yields averaged 4.9 Mg/ha compared to 7.2 in 1982 and 8.0 in 1986 (12). The individual plot data for this study were obtained through the cooperation of farmers in Wabash County, Indiana, and the USDA Soil Conservation Service (SCS) during 1983. The *Soil Survey of Wabash County, Indiana* (11) provided much of the background information on soils.

Methods and Materials

Primary data for this study were gathered in 78 plots located in farmers' fields. Plots within fields were chosen to be homogeneous and representative of a particular Indiana soil type by SCS soil scientists. Observations were made on soil texture, erosion class, slope, depth of root zone, and primary soil classification for each plot. Soil samples were taken for laboratory analysis from each plot during the fall of the year when the corn was harvested to obtain yield estimates. A weediness rating for each plot based on the procedure outlined in *The Field Crops IPM Scout Manual* (3) was assigned at the same time. The farmers also furnished information on the amount of fertilizer applied, planting date, crop sequence, pesticide usage, and method of tillage used. Rainfall data were measured for each field from April 4 through June 26, and for 12 locations in Wabash County through September 15. The daily precipitation record for each of the field locations was completed from that measured at the nearest of the 12 stations. A summary of the data for the more important variables is included in the appendix.

Data and Analysis

The corn yields on the plots ranged from 0.4 to 10.0 Mg/ha (7 to 160 bu/ac) during 1983. The mean corn yields by tillage system for 1983 are shown in table 1. The mean yields, as well as an analysis of variance of only the yield data, in-

TABLE 1. Means of individual plot data for corn by tillage system.

	Conventional	Ridge till	No-till
	.....previous crop—corn-----		
Observations	16	7	9
Yield (Mg/ha)	3.4	6.2	3.4
Stress	25.2	20.4	30.7
Nitrogen (kg/ha)#	166.2	190.3	202.4
Phosphorous (kg/ha)&	127.5	125.3	137.6
	————previous crop—soybeans-----		
Observations	9	16	21
Yield (Mg/ha)	4.2	5.6	4.5
Stress	27.3	25.0	27.1
Nitrogen (kg/ha)#	200.1	233.4	192.4
Phosphorous (kg/ha)&	141.5	131.2	158.6

Nitrogen applied; plot application increased by 22.4 kg/ha for corn in rotation with soybeans to account for nitrogen fixation of the legume crop.
& Available phosphorous based on soil test results.

dicated that the ridge system had a distinct yield advantage over the no-till and conventional systems. Acceptance of these results without consideration of other inputs could lead to a conclusion that the ridge system is far superior to the other systems for the kind of weather patterns experienced in Wabash County during 1983.

Many other factors, however, interact to affect corn yield. These factors include rainfall received, solar radiation, potential evapotranspiration, soil water holding capacities, natural drainage conditions, fertilizer application rates, cropping sequence, and planting data. The yields reported in table 1 are not adjusted for the influence of any of these factors. Because of the summer heat and lack of rainfall during 1983, moisture stress was suspected to be the most limiting factor in determining corn yields and the principal cause of the variability in final yields observed among plots.

Variables affecting corn moisture stress include soil water holding capacity in the corn root zone, rainfall, potential evapotranspiration, and corn development. The program, SIMBAL, SIMulation of soil water BALance on poorly-drained and well-drained soils (1, 2, 10), was used to incorporate soil and daily weather information over the growing season into a single measure of plant stress. Moisture stress is defined as [1 - ET/PET] where ET is the actual plant evapotranspiration and PET is potential evapotranspiration, a measure of the atmospheric evaporative demand. ET depends both on the plant-available soil water supply and PET. If soil moisture is not limiting, ET is equal to PET (1 - ET/PET = 0), and the corn plant experiences no moisture stress. If ET is less than PET, the corn plant suffers moisture stress. Moisture stress was calculated daily for the entire growing season for each farm plot with SIMBAL.

Using an energy-crop growth variable which includes ET/PET, (7) found the period most highly correlated with final corn yield from 40 days before (and including) silking to 50 days after. Therefore, the variable used to identify mositure stress was the summation of the daily (1 - ET/PET)for this 90-day period for each corn plot.

Because potential evapotranspiration is relatively stable from year to year, precipitation and plant-available soil water holding capacity (WHC) are usually the two most important variables in determining moisture stress. The problem with evaluating tillage (or any management system) without considering spatial variability is demonstrated visually in figures 1 and 2. Three categories of WHC, total summer precipitation, and the 90-day moisture stress summed for each plot are shown in figure 1. We should point out that the "high, medium, and low" groupings for precipitation in figure 1 are relative to the 1983 average precipitation measured at various locations which, at the Wabash weather station, was about 78% of normal. Although there is considerable spatial variability in the data as reflected on the maps, note that during 1983 the patterns of total precipitation and WHC were similar, a random occurrence which tended to increase the range of moisture stress and corn yields between soils with lower and higher WHC. We should also point out that it is the distribution of the precipitation during the summer which is important for corn yield, not just the total. The distribution of precipitation is considered in the 90-day stress summation.

The three tillage systems used by farmers on the individual plots and three classes of final corn yields are plotted in figure 2. As with precipitation, the "high, medium, and low" yield classes are relative to 1983 and were considerably below normal as previously indicated. The tillage systems evaluated were conventional, ridge till and no-till. The conventional system is broadly defined and includes both moldboard plowing and chisel plowing followed by disking in the spring; the ridge till and no-till definitions are for standard practices (4). Some spatial groupings of tillage systems occur by soils. The ridge system tends to be used on soils with high WHC and poor drainage while the no-till system tends to be used on well-drained soils with lower WHC, both observations in accord with university recommendations.

The tendency for the weather and soil variability to confound the tillage treatment interpretations is demonstrated visually in figures 1 and 2 by the dashed lines marking three groups of selected plots, one for each tillage treatment. The no-till group is A (4 plots), B is a group of ridge till plots (7 plots), and C is two plots with conventional tillage. For A, the circled no-till plots (figure 2) generally have low WHC, "medium" precipitation, and high stress (figure 1), leading to low yields (figure 2). The circled ridge till plots (group B) had high WHC, "high" precipitation, and low stress which led to "high" yields in figure 2 and certainly contributed to the "significant" ridge till yield performance in table 1. For group C, the conventional tillage plots had low to medium WHC and precipitation, high stress, and resulting low corn yields.

The plot means for stress summation, nitrogen fertilizer applied, and available soil phosphorous are also listed in table 1 by tillage system. Mean moisture stress is lower for the ridge plots than the other tillage plots as indicated in figures 1 and 2. Based on Purdue Soil Testing Laboratory recommendations (9), fertilizer nitrogen rates for plots where the previous crop was soybeans were adjusted upwards by 22.4 kg/ha (20 lbs/ac) to account for the nitrogen fixation of the preceding soybean crop. There is no distinct relationship between either nitrogen or phosphorous and tillage system.

Linear regression techniques were used to correct for differences in inputs among plots by estimating quadratic response functions for corn yield. Independent variables

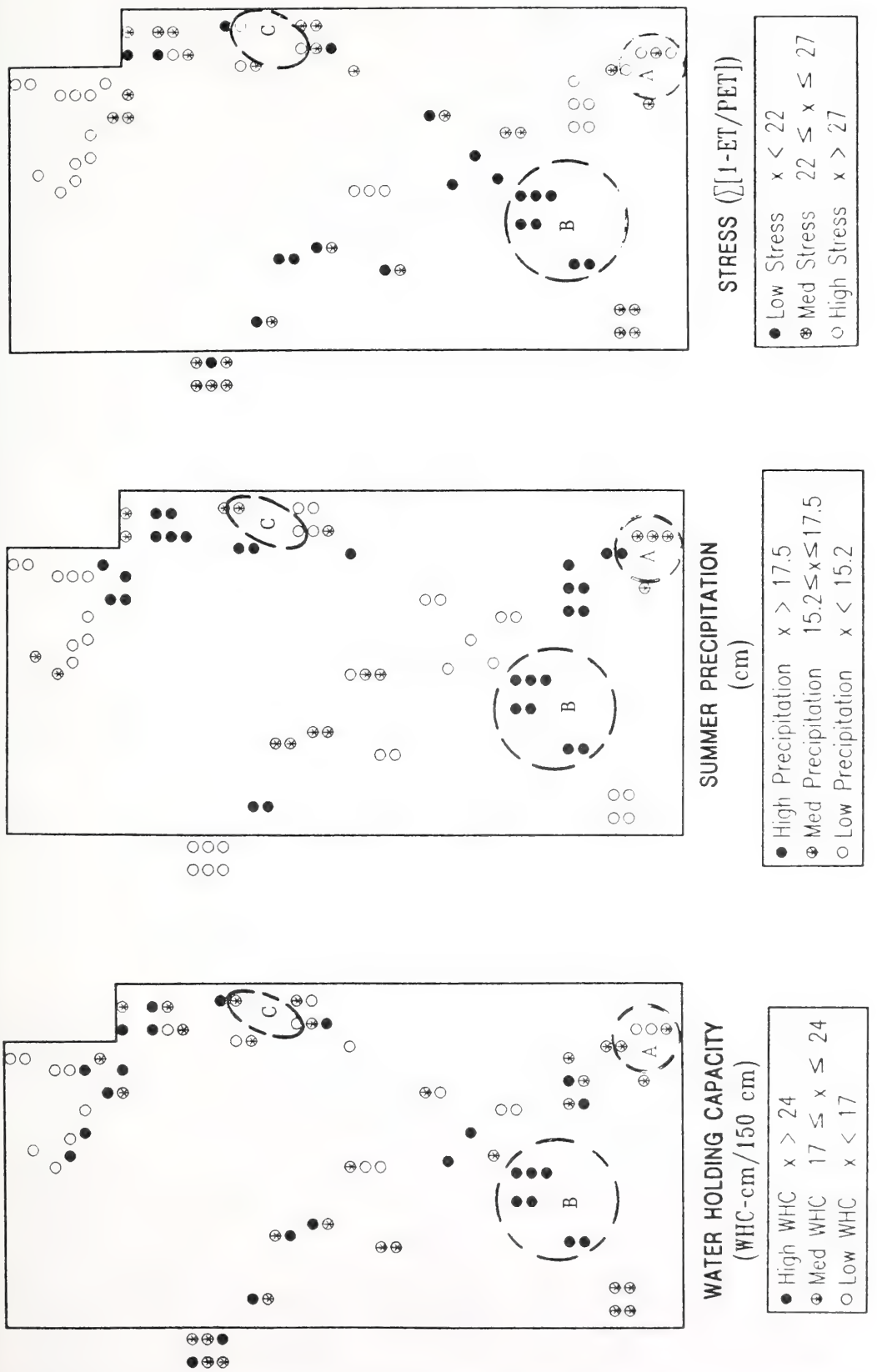

FIGURE 1. Spatial representation of soil water holding capacity, summer precipitation, and estimated moisture stress by plot, Wabash County - 1983.

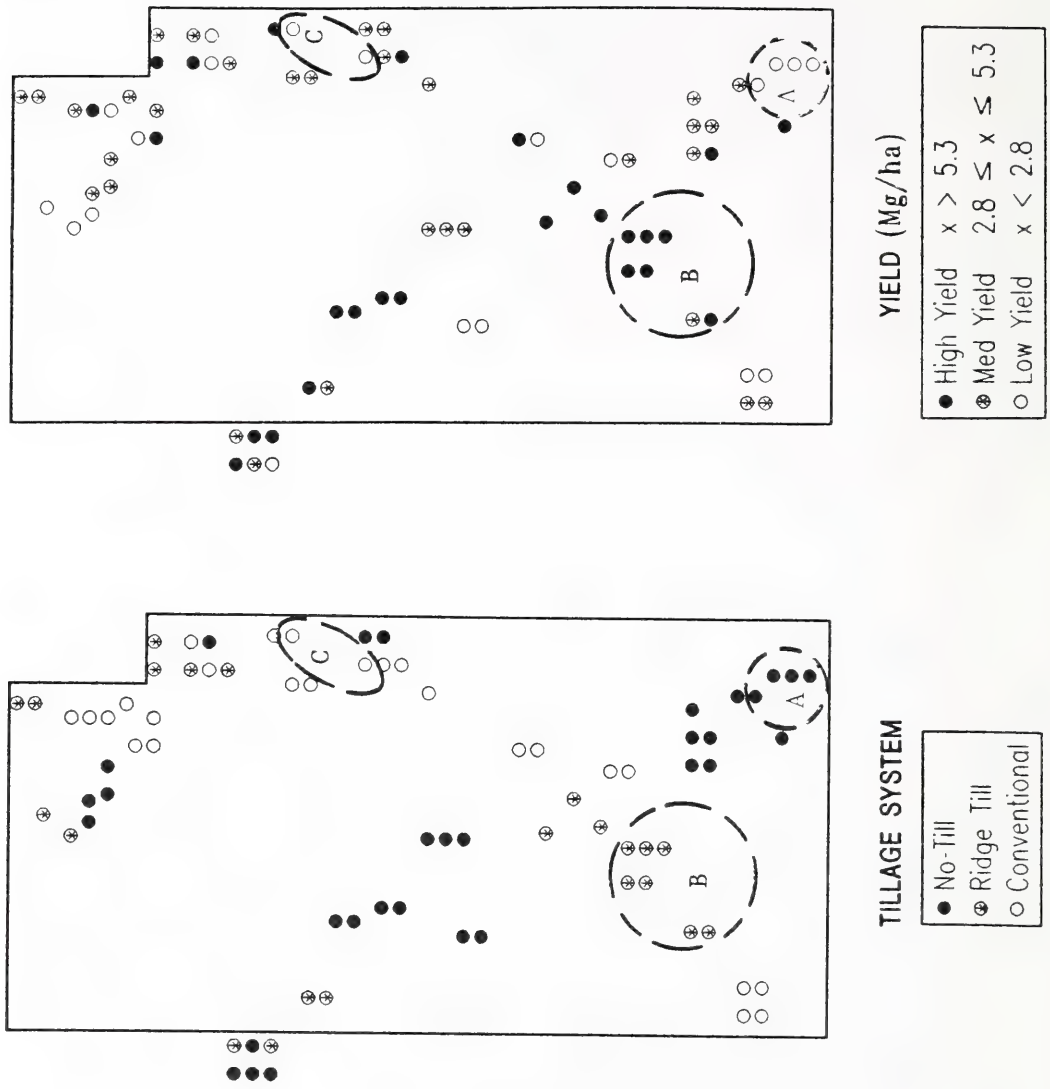

FIGURE 2. Spatial representation of tillage system and corn yield by plot, Wabash County - 1983.

used to estimate final corn yields (Y) were: both linear and quadratic terms for stress (S), nitrogen (N), and phosphorous (P); a binary variable for previous crop (PC); binary variables for the no-till (NT) and ridge-till (RT) systems; and no-till*stress (NT*S) and ridge till*stress (RT*S) interaction terms. Tillage system*stress interaction terms were included to consider differing corn yield responses to estimated moisture stress by tillage system. A N*S interaction as well as the weediness rating were both considered in early analyses, but the significance of each was low. Therefore, neither term is considered in the analysis presented.

Although the no-till and ridge till systems may conserve soil moisture more than conventional tillage (5), we should recognize that the development of the soil moisture model and resulting SIMBAL-generated ET/PET calculations are based on experimental findings on conventionally tilled soils. Thus, the interaction terms reflect only the yield effect stemming from the joint occurrence of the tillage system used with the calculated moisture stress based on the respective WHC and weather data.

Results

The regression coefficients for the model considered,

$$Y = b_0 + b_1S + b_2S^2 + b_3P + b_4P^2 + b_5N + b_6N^2 + b_7PC + b_8NT + b_9NT*S + b_{10}RT + b_{11}RT*S, \quad [1]$$

are shown in table 2 with variables defined. All but the intercept, the ridge till binary,

TABLE 2. Fitted regression coefficients for corn yield (Mg/ha) response [1] estimated from data obtained from Wabash County, Indiana farmers, 1983.

Variable	Coefficient#
Intercept	7.16
	(1.21)
S, Stress	-1.60*
	(-6.28)
S^2	0.0215**
	(5.15)
P, Phosphorous (kg/ha)	0.0314**
	(2.63)
P^2	-0.000105**
	(-2.81)
N, Nitrogen (kg/ha)	0.22**
	(3.80)
N^2	-0.000556**
	(-3.78)
PC, Previous crop (O = corn, 1 = soybeans)	1.47**
	(3.21)
NT, No-till binary (NT = 1; RT, Conv = 0)	-5.34*
	(-1.84)
NT*S, No-till*stress (interaction)	0.21*
	(1.99)
RT, Ridge-till binary (RT = 1; NT, Conv = 0)	-3.36
	(-1.25)
RT*S, Ridge-till*stress (interaction)	0.11
	(1.08)

R-square - 0.66, Adj. R-square - 0.60
t values in parenthesis
** significant at one percent level
* significant at ten percent level

and the RT*S interaction variables are significant. The no-till binary and the NT*S interaction are significant at the ten percent level; the rest are significant at the one percent level. The F value for the regression (11.4 for 11 and 66 d.f.) was highly significant although the adjusted R^2 was only 0.60.

In 1983, moisture stress had much greater effect on corn yields than did nitrogen *within the 100-200 kg/ha* range used by the farmers. The phosphorous results also indicate a flat relationship. That is, although the coefficients were significant, corn yield was affected very little as the N and P levels changed within the fairly optimum range used by the farmers.

The regression results indicate that corn planted after soybeans yielded 1.47 Mg/ha (23.5 bu/ac) more than corn after corn, a higher estimate than is normally suggested, especially since some of the legume effect is included in the N variable. Some agronomists hypothesize that the allelopathic effect is larger in dry years, thus this estimate is possible given the extreme conditions of 1983 (8).

In the range of the stress variable reflected by the 1983 observations, as stress increased, corn yield decreased at a decreasing rate. Relationships between stress and yield are plotted in figure 3 for the three tillage systems evaluated for corn following

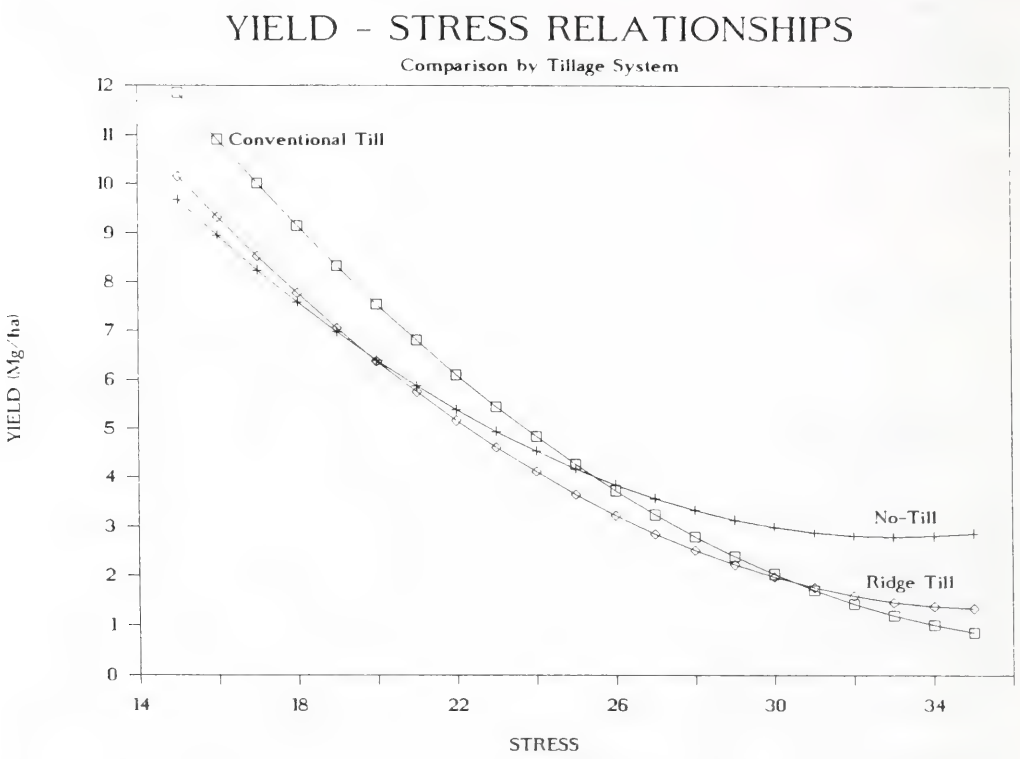

FIGURE 3. Regression model ([1], table 2) of corn yield on stress for indicated tillage system for corn following corn at average fertility levels (N = 200 kg/ha, P = 140 kg/ha). Wabash County - 1983.

corn (PC = O) and the average fertility levels observed on the plots in the study, approximately 200 kg/ha of applied nitrogen and a phosphorous level of 140 kg/ha.

None of the tillage system coefficients were significant at the one percent level of confidence, but the no-till and NT*S coefficients are significant at the 10 percent level of confidence. The three fitted curves in figure 3 show dramatic yield decreases with increasing stress compared to the differences between tillage systems. At the mean level of stress in 1983 (26), the no-till system yielded only 0.1 Mg/ha less than the conventional systems. But as stress increased, the no-till system became more attractive relative to the conventional system. In fact, if moisture stress were greater than 27, estimated no-till yields are greater than those on conventional plots. With little stress, however, conventional yields were higher. Such results suggest the effect of the tillage*stress interactions, although we should caution that the 95% confidence limits on the mean yield predicted for each of the three tillage systems in figure 3 overlap.

Concluding Comments

The hypothesis, suggested by the initial averages, that ridge till corn yields were significantly higher than corn yields under the conventional system cannot be accepted using 1983 Wabash County data. We conclude that no significant differences in *mean* corn yields due to tillage system remain after adjustments are made for differing levels of other inputs. The most important input in explaining the initial difference in corn yields was moisture stress. The results do suggest, however, that corn yields under both the no-till and ridge till systems improve relative to conventional tillage as the moisture stress experienced by the corn plant increases. The results lend some support to the hypothesis that no-till has some yield advantage over both the ridge and conventional tillage systems in extremely droughty conditions.

Note, however, that the tillage coefficients are not as highly significant as others in the regression and that the study only reflects results from a single year characterized by considerable spatial weather variability within a single county. While we believe the results are in the proper direction, additional research is necessary to better understand the complex relationships among plant growth and final yield with climatic factors, soil properties, fertility, and tillage system.

Acknowledgments

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The cooperation of the Wabash County farmers who worked with us on this study is appreciated. We also wish to thank Vince Harrell, the Wabash County Cooperative Extension Agent; Mel Boyer, former SCS District Conservationist; Don Ruesch, SCS Area Soil Scientist; and Ray Sinclair, SCS State Soil Scientist. Their patience and interest in this project are much appreciated. We also appreciate the cooperation of other scientists at Purdue University. Bill Moldenhauer (ARS/USDA-retired); Don Griffith, Jerry Mannering, and Dave Mengel (Dept. of Agronomy); and Sam Parsons (Dept. of Ag. Engineering); all provided valuable input into this research. We also appreciated Jerry Mannering's review of an earlier version of this paper.

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APPENDIX. Individual plot data for variables used in regression model [1] or plotted in figures 1 and 2. Wabash County study—1983.

Yield Mg/ha	Stress	Water Holding Capacity cm/150cm	Summer Precip cm	Tillage# System	Previous& Crop	Applied* Nitrogen kg/ha	Soil Phosphorus kg/ha
3.06	25.3	16.2	14.4	C	C	169.1	104.2
2.51	26.0	16.5	14.4	C	C	169.1	65.0
7.22	15.7	25.3	18.0	R	C	213.9	152.3
9.64	15.8	24.7	18.0	R	C	213.9	98.6
4.08	23.6	20.7	12.8	R	S	208.3	107.5
3.75	29.6	15.8	20.7	C	S	189.3	32.5
6.68	20.7	23.2	13.3	N	S	253.1	185.9
6.79	22.3	25.3	12.8	R	S	208.3	96.3
4.43	26.7	17.2	20.7	C	S	189.3	23.5
5.96	24.4	25.3	12.8	N	S	230.7	197.1
4.34	26.7	20.7	12.8	N	S	230.7	140.0
7.52	22.6	25.3	17.8	R	S	228.5	224.0
4.55	23.3	20.6	17.8	R	S	228.5	82.9
1.88	24.7	25.6	13.3	N	S	253.1	161.3
5.64	21.8	25.6	18.3	R	C	189.3	174.7
3.79	23.4	25.6	18.3	C	C	142.2	156.8
4.50	24.5	20.1	18.3	N	C	142.2	65.0
1.04	30.2	15.8	18.3	C	C	189.3	109.8
3.22	26.1	18.0	18.3	R	C	189.3	129.9
4.69	24.3	18.0	14.2	N	S	161.3	44.8
3.48	23.4	16.5	14.2	N	S	161.3	49.3
2.13	27.6	14.8	14.2	C	C	142.2	96.3
4.58	25.8	18.0	14.2	C	C	142.2	113.1
6.00	21.1	26.5	17.1	C	C	142.2	107.5
3.46	23.5	16.5	19.1	C	C	157.9	268.8
3.30	34.9	12.8	13.7	N	S	188.2	268.8
3.21	30.8	24.4	12.8	N	S	188.2	268.8
3.48	44.5	16.0	13.8	R	S	219.5	268.8
1.61	33.9	9.8	17.0	R	S	237.4	116.5
3.86	40.6	9.4	13.8	R	S	219.5	88.5
5.03	34.9	13.9	13.7	N	S	188.2	268.8
0.98	30.5	25.8	12.8	N	S	188.2	268.8
3.14	35.5	11.7	13.8	C	S	201.6	156.8
2.09	31.0	11.3	13.8	C	S	201.6	268.8
5.51	28.6	29.9	13.8	C	S	201.6	155.7
1.60	32.5	10.7	17.0	R	S	237.4	191.5
4.29	26.8	19.1	15.7	R	S	213.9	50.4
8.46	22.2	25.6	15.7	R	S	213.9	60.5
4.08	25.6	25.5	18.5	C	C	190.4	174.7
7.71	21.2	25.3	16.6	N	S	216.2	203.8
6.98	20.8	27.6	16.8	C	S	164.6	268.8
1.89	27.7	18.0	16.8	C	S	164.6	257.6
8.63	21.2	25.3	17.3	N	S	216.2	197.1
6.96	21.6	22.3	16.6	N	S	216.2	73.9
4.66	37.3	17.8	13.5	N	C	246.4	129.9
2.93	28.4	20.4	18.5	C	C	190.4	216.2
6.99	24.4	20.9	17.3	N	S	216.2	107.5
0.71	25.2	25.5	18.5	C	C	190.4	268.8
5.51	26.4	20.7	18.5	C	C	190.4	144.5
4.65	40.0	16.3	17.2	N	C	179.2	90.7
3.11	39.7	16.8	17.2	N	C	179.2	104.2
8.56	21.7	18.0	13.1	C	S	244.2	60.5
1.57	23.7	16.0	13.1	C	S	244.2	52.6
4.80	33.1	18.0	18.5	N	S	183.7	136.6
6.13	29.2	25.6	18.5	N	S	183.7	129.9

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APPENDIX. Individual plot data for variables used in regression model [1] or plotted in figures 1 and 2. Wabash County study—1983.

Yield Mg/ha	Stress	Water Holding Capacity cm/150cm	Summer Precip cm	Tillage# System	Previous& Crop	Applied* Nitrogen kg/ha	Soil Phosphorus kg/ha
4.08	29.3	25.6	18.5	N	C	161.3	133.3
3.44	33.4	19.1	18.5	N	C	161.3	248.6
3.04	36.0	17.5	18.5	N	S	183.7	52.6
6.48	15.3	25.0	13.4	R	S	274.4	73.9
9.10	17.4	25.0	18.0	R	S	250.9	268.8
7.36	17.9	26.5	18.0	R	S	250.9	57.1
7.00	16.2	29.9	18.0	R	S	250.9	248.6
6.49	17.2	22.3	14.5	R	C	168.0	104.2
10.05	15.9	27.7	14.5	R	C	168.0	107.5
4.61	23.7	20.1	20.9	N	C	198.2	78.4
1.89	27.5	18.6	20.9	N	C	198.2	54.9
1.43	30.9	15.8	17.0	N	S	145.6	209.4
2.06	25.8	15.4	17.0	N	S	145.6	109.8
2.49	27.4	18.0	17.0	N	S	145.6	57.1
5.73	23.7	19.2	17.0	N	S	146.6	69.4
1.88	22.1	20.6	13.5	N	C	136.6	129.9
2.08	23.2	19.2	13.5	N	C	136.6	268.8
5.09	20.4	26.4	18.5	R	S	246.4	90.7
7.47	20.4	27.7	18.5	R	S	246.4	71.7
3.55	26.5	21.9	14.2	C	C	172.5	44.8
4.73	23.7	21.9	14.2	C	C	172.5	107.5
1.86	25.9	20.9	14.2	C	C	172.5	59.4
0.46	24.5	18.6	14.2	C	C	172.5	47.0

C - conventional tillage, R—ridge till, N—no-till

& C - corn, S - soybeans

* Nitrogen applied; plot application increased by 22.4 kg/ha for corn in rotation with soybeans to account for nitrogen fixation of the legume crop.

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Some Properties of Glacial Till and Till-derived Soil Horizons in East Central Indiana

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Introduction

Indiana recently completed a 10-year accelerated soil survey program which resulted in every county in the state being mapped. During this time of intensive study, soil scientists gained considerable knowledge about most of the soils which enabled them to make more accurate soil interpretations. Some series concepts have been narrowed to better reflect these interpretations which have made soil surveys more useful to farmers, homeowners, land use planners and other users of soils information. As a consequence of defining soil series more specifically, some similar soils were assigned different names in nearby counties. Now that the field work has been completed for all the modern surveys we have the opportunity to integrate the information in the individual surveys by examining the geography of soils from a regional viewpoint, rather than by counties, and by using the same definitions for all soils.

In the glaciated region, many soil differences are related to the nature of the glacial till on which they formed. To learn how the till varied regionally, Franzmeier et al. (1985) summarized existing information about properties of the till by geographic areas. That summary showed that there could be major differences among the tills of east-central Indiana where several glacial lobes came together. Most of the till of the region was deposited by the East White sublobe of the Ontario-Erie glacial lobe, but the eastern part of it (areas 6 and 8 in Franzmeier et al.) was deposited by the Miami sublobe. However, the number of observations in this area was too small to characterize the patterns accurately.

The objectives of this study were to run additional transects and analyses (1) to characterize the geographic differences, if any, in several properties of the glacial tills in east-central Indiana, (2) to characterize the geographic differences in the clay content of the till-derived B horizons, and (3) to examine the relationship to the clay content of the B horizons to that of the underlying till.

Methods and Materials

Two transects were laid out through Madison, Hancock, Delaware, Henry, Randolph, and Wayne Counties (Figure 1). These transects are all in the Cartersburg Till member of the Trafalgar formation (Wayne, 1963). The transect region had been subdivided into five areas in the previous study (Franzmeier et al., 1985) to look for possible differences in till due to the origin of the glacier that deposited it.

Along each transect a potential site was located on the summit position of the local landscape in every section. This site was sampled if the soil were formed in till and if permission could be obtained from the property owner. Most of the sites

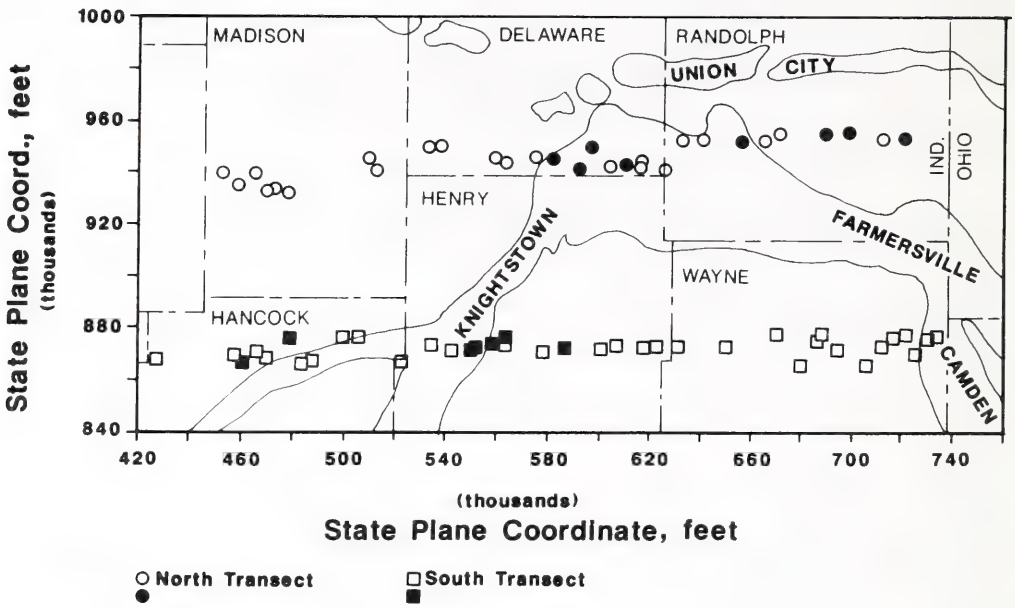

FIGURE 1. Map of east-central Indiana showing the locations of the moraines and sampling sites in the Indiana state plane coordinate system, east zone. The solid symbols represent the sites in which the glacial till contained more than 20% clay.

were on nearly level landscape swells with a thin (< 20 inches) loess cap. The soils at these sites were in the Crosby series (Aeric Ochraqualf, fine, mixed, mesic). Some sites were on gently sloping steeper summits, had a thinner loess cap, and were well drained. These soils were mainly in the Miami series (Typic Haplaudalf, fine-loamy, mixed, mesic).

Samples were collected with a truck-mounted hydraulic probe to a depth a few feet into the calcareous till. The core samples were wrapped in freezer paper in the field and described and subsampled a few days later indoors because of rainy weather at sampling time. Samples were taken of the unaltered till, usually about 12 to 20 inches below the upper boundary of calcareous material, and of the major portion of the B horizon formed from till. If none of the B horizon were formed in till, it was not sampled. In these soils all of the B horizons formed in loess, in inclusions of outwash, or in both of these materials. Bulk samples were collected from B and C horizons. In addition an undisturbed section of the core of the C horizon was coated with Saran resin for determination of bulk density. The average depth of sampling of the C horizon was 46.2 inches across both transects.

The bulk samples were dried, crushed, and passd through a 2-mm sieve. Bulk density measurements were corrected to the <2-mm basis by subtracting the weight and volume of coarse fragments from the measurements made on the whole sample. It was assumed the coarse fragments had a density of 2.65 g/cm^3. Particle-size distribution was determined on all samples by the pipet method. In addition C horizon samples were analyzed for calcium carbonate equivalent (CCE) by acid neutralization. The procedures are described in detail in Franzmeier et al. 1977.

Results and Discussion

For both transects the <2-mm glaical till contained an average of 38% sand, 44% silt, and 18% clay (Table 1) that placed it near the center of the loam texture class. It is very similar to the till on the Camden moraine and north of it to the

TABLE 1. Average particle-size distribution of B and C horizons.

Horizon	Coarse Fragments	vcS	Particle-Size Class cS	ms	fS	vfS	cSi	fSi	Total C	Total Si	Total S	Number of Samples
			% of < 2mm									
North transect												
B		1.2	2.2	4.9	10.3	6.7	11.4	25.9	37.4	37.3	25.3	24
C	12.6	4.4	5.1	6.8	12.2	8.7	17.2	27.0	18.6	44.2	37.2	30
South transect												
B		1.5	2.9	6.4	11.6	7.1	10.3	23.3	36.9	33.6	29.5	33
C	9.6	4.1	5.8	8.2	12.8	8.5	18.2	24.9	17.5	43.1	39.4	38

Union City moraine in Ohio, which contained 42% sand, 40% silt, and 18% clay (Steiger and Holowaychuk, 1971). For the same till in Indiana, however, Gooding (1973) reported 37% sand, 40% silt, and 23% clay. The till we studies averaged around 40% calcium carbonate equivalent, and nearly 2.0 g/cm^3 bulk density (Table 2).

TABLE 2. Average bulk density and calcium carbonate equivalent of C horizons.

Transect	Bulk Density Whole Soil	Bulk Density < 2mm	Number of Samples	Calcium Carbonate Equivalent CCE	Number of Samples
	g/cm^3			%	
North	1.99	1.94	22	40.5	30
South	2.01	1.96	24	41.1	38

The clay content of the C horizon increased slightly from west to east in the north transect, but over all, no geographic trends were noted (Figure 2). There appeared to be a natural separation between those sample with more than 20% clay and those with less. In order to learn if the higher-clay tills were related to some geographic feature, the locations of sites in which the soil contained more than 20% clay were plotted on a map (Figure 1) using filled circles and squares. The open symbols in that figure represent samples with <20% clay. The high-clay sites were fairly randomly located, except that most of them were on or near the end moraines. In Figure 1 the location of the named moraines is from Wayne (1965). The moraine across Hancock County was taken from an old map by Mallott (undated). It was not mapped by Wayne.

Calcium carbonate equivalent (CCE) also showed no trends across the transects (Figure 3). Most of the samples had 35 to 45% CCE. Bulk density, likewise, showed no trends (Figure 4). The values were very high, mainly between 1.9 and 2.1 g/cm^3.

The clay contents of the Bt horizons mainly ranged from 30% to 45%, but showed no geographic trends (Figure 5). Most of the samples with more than 40% clay are scattered in the central part of the study area (Figure 6). The average Bt horizon was near the center of the clay loam class (Table 1) and contained 37% clay, more than twice as much as the average C horizon. In a similar soil in Ohio, Smeck et al. (1968) attributed most of the clay increase in the Bt horizon to loss of carbonates. In the C horizon, most of the carbonate minerals were in the sand fraction and as these minerals weathered the clay percentage increased due to loss of the "diluting" coarser fraction.

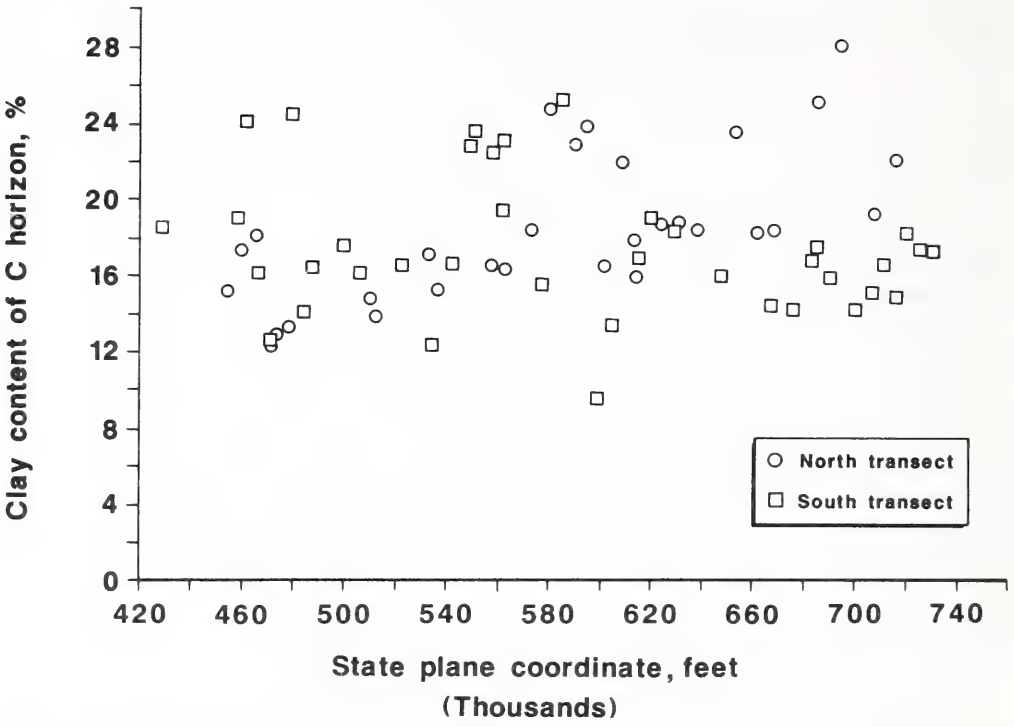

FIGURE 2. Relation of the clay content of glacial till C horizons to distance along the transects.

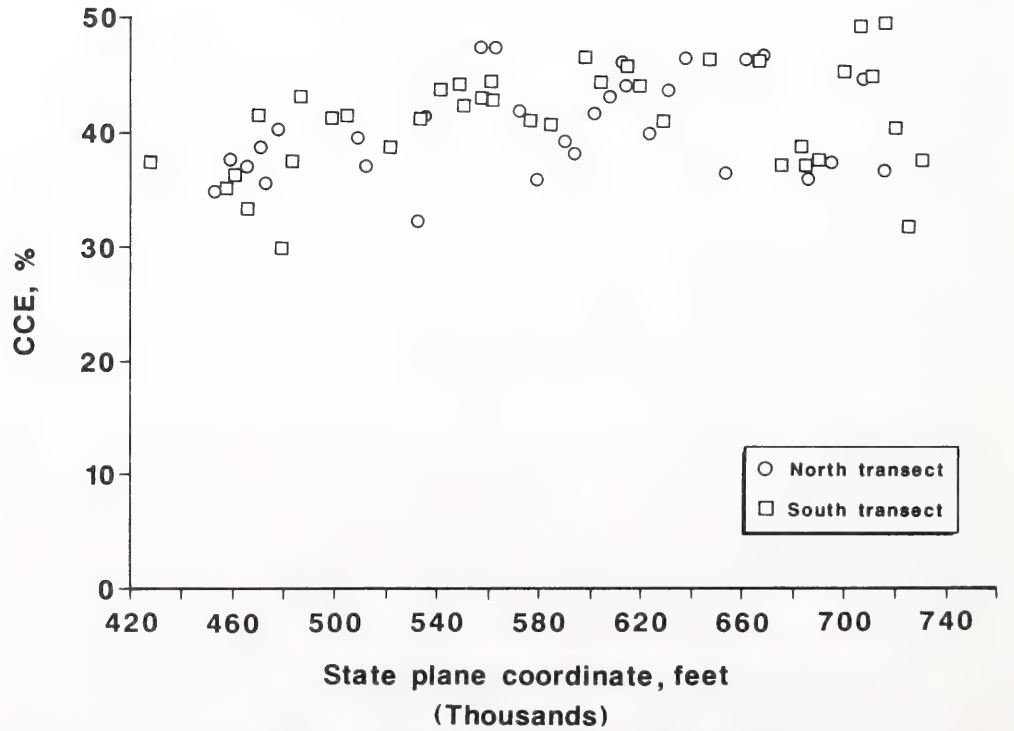

FIGURE 3. Relation of the calcium carbonate equivalent (CCE) of C horizons to distance along the transects.

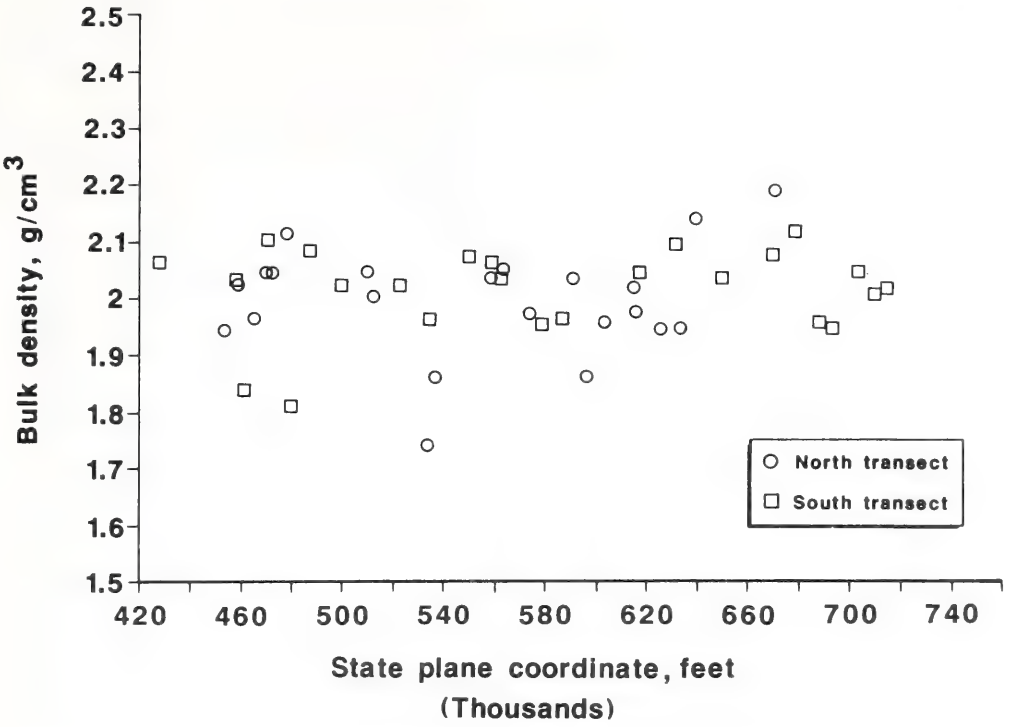

Figure 4. Relation of bulk density of C horizons to distance along the transects.

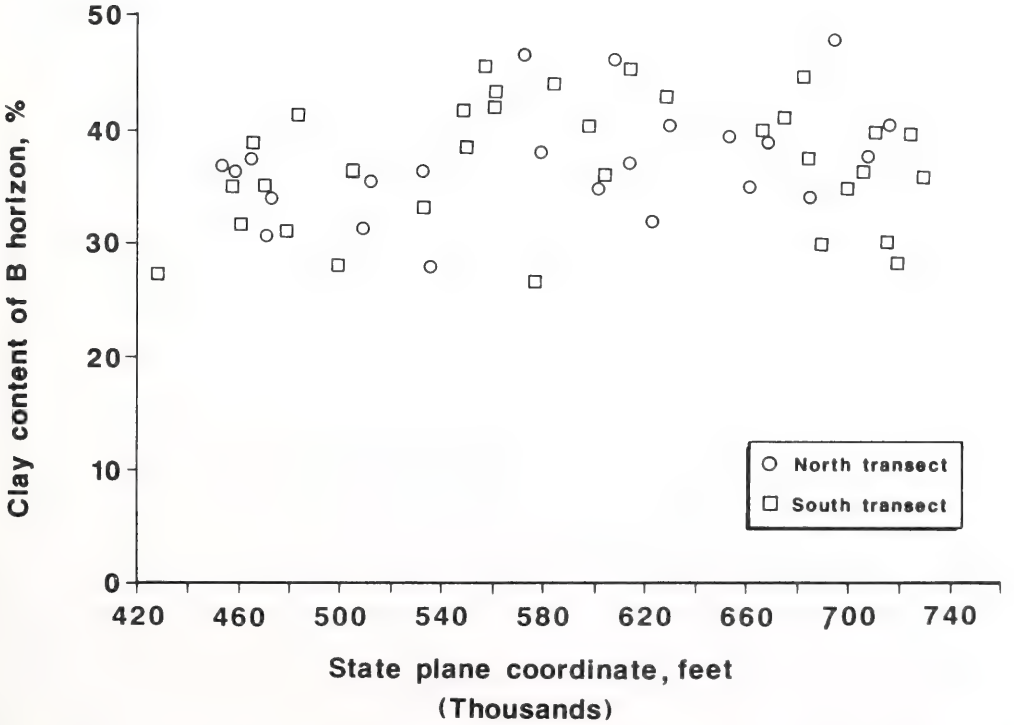

Figure 5. Relation of the clay content of till-derived Bt horizons to distance along the transects.

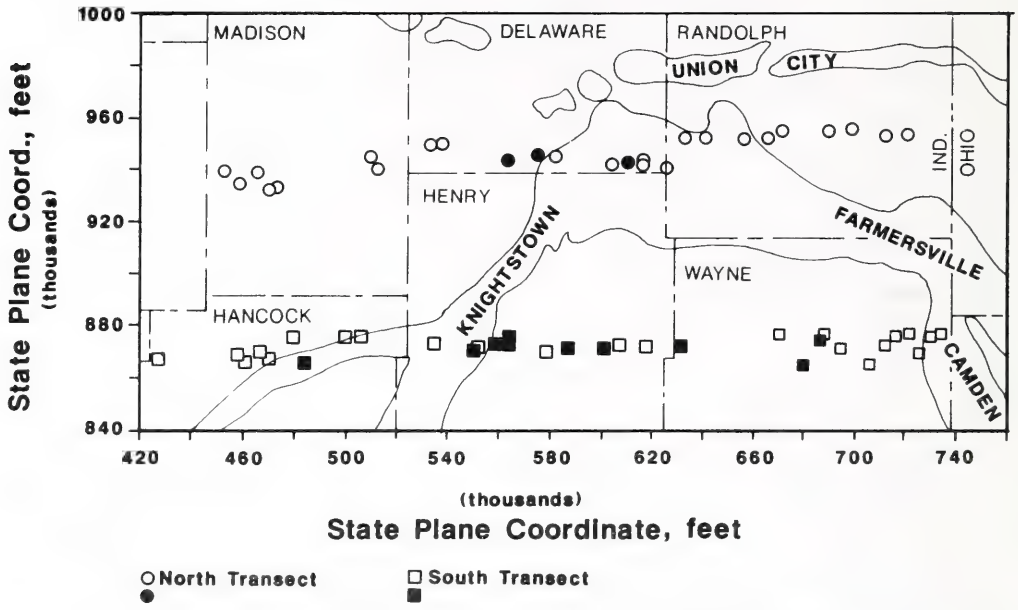

FIGURE 6. Map showing the location of soils in which the Bt horizons were sampled. The solid symbols represent the soils in which the Bt horizons contained more than 40% clay.

The data were also examined to look for possible relationships between the clay contents of the B and C horizons (Figure 7) assuming the other four soil-forming factors—microclimate, topography, vegetation, and time—were similar. There was a slight trend for the clay content of the B horizons to increase with increasing clay in the C horizons ($Clay_B = 29.4 + 0.43 \times Clay_C$, $r = 0.32$). There was insufficient range in the data to obtain the higher correlation reperted by Franzmeier et al. (1985) for a wider range of glaical tills. It is also possible that the B horizons developed in material different from the present-day C horizon. Wayne (1968) reported that in much of the till plain in east-central Indiana the surface deposit consists of loose till mixed with thin layers of crudely water-worked sediments which could be called ablation till. This material could be the parent material of the B horizons. The C horizons, on the other hand, would not be considered to be "loose material" because they have a bulk density of around 2.0 g/cm^3 and have very firm consistence. Morphological evidence also suggests that soil formation does not extend very far into dense till.

The good correlation between clay content of B and C horizons across areas of diverse tills suggests that the upper till layer, from which the Bt horizons formed, and the lower till are generally similar, probably because they were derived from similar sources. This relationship is on a scale compatible with showing relations across the state. The same relations, however, may not apply at a smaller scale, such as a county or a few counties, if the differences in the till are not great.

The data also shed some light on the particle-size classification of the Crosby series, the dominant one sampled. The average clay content of the Bt horizons is 37% (Table 1). This is very near the 35% that separates fine from fine-loamy classes in Soil Taxonomy. The zone sampled to represent the major Bt horizons was thinner and probably contained more clay than the family control section zone, so the control section probably contained somewhat less than 37% clay, even closer to the 35% limit. Thus the Crosby series, as a natural soil-landscape body, is fairly homogeneous,

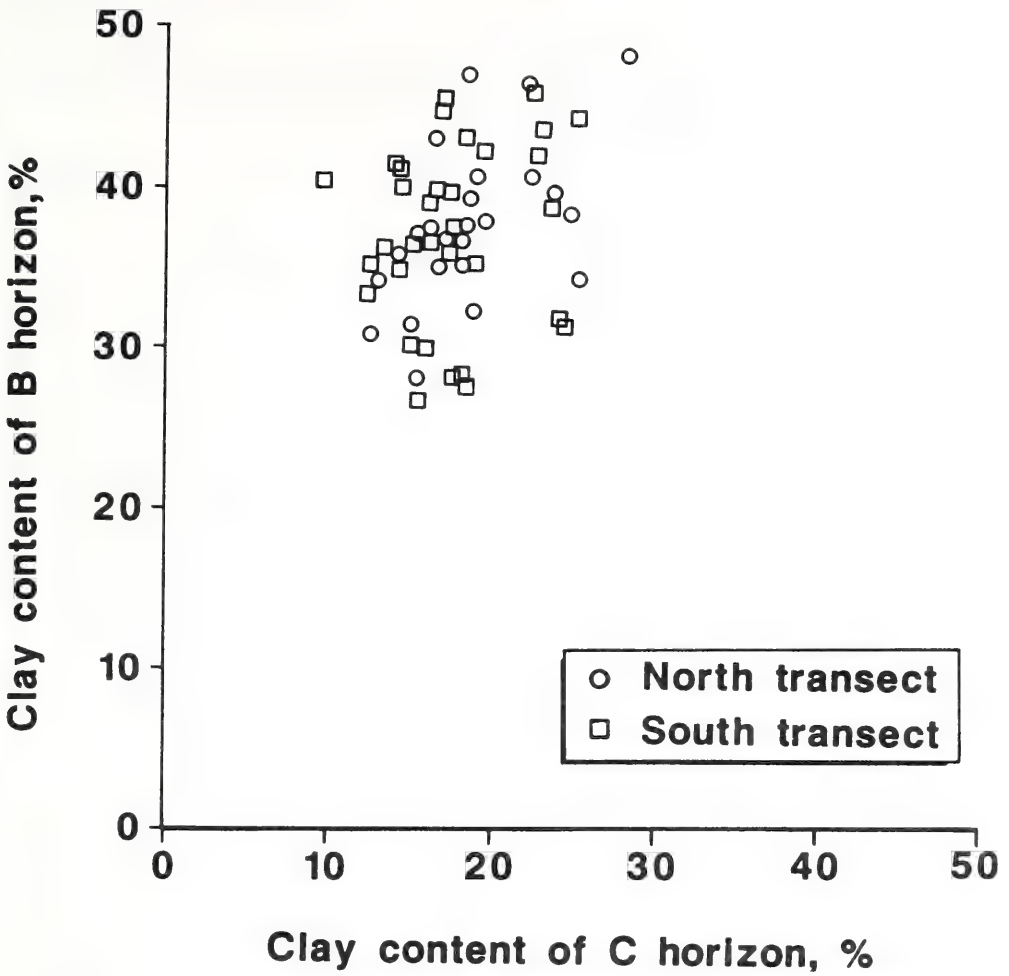

Figure 7. Relation of the clay content of the Bt horizons to the clay content of the C horizons.

but it straddles a soil taxonomy boundary. It is not possible to map soils using this separation, so the Crosby series is classified as fine, but the map units are about half fine-loamy.

Conclusions

The Cartersburg till of east-central Indiana is relatively uniform. On the average it contains 38% sand, 44% silt and 18% clay, and it has a calcium carbonate equivalent of 41% and bulk density of 2.0 g/cm^3. There is some variability among the samples but it is random across the study area. It is probably caused by local factors rather than regional ones. The clay content of the Bt horizons also shows a random geographical distribution. It is poorly correlated with the clay content of the C horizons probably because the two horizons formed in different materials. The Bt horizons appeared to have formed in less dense till that was deposited over the more dense till. This upper till material, however, was most likely derived from the same source as the lower material.

Acknowledgment

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Atmospheric Wet Deposition Relationships to Season and Precipitation in South Western Indiana

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Introduction

The influences of acidic precipitation on the environment has been in the forefront of discussion across the United States for at least the past ten years. Precipitation at four sites within the state of Indiana has been collected for chemical analysis since 1984 as part of the National Atmospheric Deposition Program (NADP). Analysis of the concentrations and depositions of the various precipitation constituents helps describe the atmospheric environment and the impact the environment has on the ground surface chemistry. Of particular interest is the amount, variability and sources of acidity within the precipitation, as well as the climatology of the storm systems depositing the precipitation.

A description of the results for the NADP site at the Purdue Agronomy Farm near West Lafayette, Indiana (3) indicated that the sulfate deposition was largely accounted for by oxidation reactions on sulfur dioxide and that both sulfate and nitrate were highly correlated with the precipitation pH. The West Lafayette site is remote from large sources of sulfur dioxide, nitrous oxides or flyash, with only one power plant (to the south of the site) within 50km (13). This study described the wet deposition from October 1984 to December 1986 at the Southwest Purdue Agricultural Center near Vincennes, Indiana which is situated in the vicinity of six power plants (surrounding the site) within 50 km (13). Both sites are classified by (13) as rural.

Methods

The Climatological Data for Vincennes, Indiana (18) were used to determine the normality of the precipitation during the period of study.

The wet deposition samples were collected according to NADP protocol (13) on Tuesday of each week. The water soluble hydrogen (H^+), sulfate (SO_4^{2-}), nitrate (NO_3^-), ammonium (NH_4^+), chloride (Cl^-), magnesium (Mg^{2+}), orthophosphate (PO_4^{2-}), and calcium (Ca^{2+}) concentrations of the wet deposition samples were analyzed by the Central Analytical Laboratory of the NADP in accordance with NADP procedures (11, 12). Precipitation amount were recorded using a Belfort weighing rain gauge.

Many sampling periods within the study period were not used due to high levels of contamination, negligible or no precipitation samples, or poor operation of the collector. If the sample had: a) no precipitation, b) precipitation less than 0.127 cm, c) PO_4^{2-} concentrations greater than 0.05 mg/L, or d) a sampler efficiency (defined as the percent of rain gauge-recorded precipitation collected in wet bucket sample) less than 75% it was not included in the data analysis. Of the initial 116 weeks included in the study period, 69 weeks passed the above criteria. These weeks were separated by season where December, January, and February defined the winter season and each season following was defined by three consecutive months (17). The 69 weeks of good wet deposition samples were distributed as 17, 15, 23, 14 in the spring, summer, fall, and winter respectively. Note that this is not a climatological series, but rather a set of precipitation periods over a 27-month period. Since the results

are based on a data set of 27 months, the overall averages are weighted unevenly with fall season precipitation contributing more data than the other seasons. Therefore the seasonal interrelationships of the precipitation chemistry are more reliable than the overall interrelationships.

Weekly precipitation was classified by amount into six categories: less than 0.5cm, 0.5 to less than 1 cm, 1 to less than 2.5 cm, 2.5 to less than 5cm, 5 to less than 10cm, and greater than or equal to 10 cm precipitation.

Concentrations reported here are weighted by the precipitation amount to adjust the relative importance of any given sample concentration to the total precipitation chemical content. For example, a precipitation weighted pH is defined as:

$$pH = \sum_{k=1}^{n} (P*pH) \; / \; \sum_{k=1}^{n} P$$

when n is the number of valid weekly samples and P is the amount of weekly precipitation.

Linear correlations between concentrations of the various chemical species and unpaired one-tailed t-tests on the differences in concentration of chemical species between seasons were made using the microcomputer version 6 of the Statistical Analysis System (microSAS).

The stepwise regression procedure of microSAS was used to determine which of the seven chemical constituents could best describe the variance in the H^+, SO_4^{2-}, NO_3^-, NH_4^+, Cl^-, Mg^{2+}, and Ca^{2+} concentrations. The criteria for entry and retention of a given dependent variable within the regression was set at an alpha of 0.05. The fraction of the independent variable variance associated with the dependent variable was defined as the partical r^2 of the final regression. The association of the variance of any constituent by any other constituent may be due to precipitation chemistry or be coincidental.

Results and Discussion

Departures from the normal (30 year average, 1951-1980) for precipitation at Vincennes, Indiana show 1984 and 1985 to be wetter than normal and 1986 to be near normal (Table 1). The fall seasons of 1984 and 1985 were very wet while the precipitation during the other seasons was within 20% of the normal. The unusually wet 1984 fall was a result of strong midlatitude westerlies with an active stormtrack through the Midwest (6). The unusually wet 1985 fall was a result of hurricane and tropical storm-related moisture (5) brought north from the Gulf of Mexico and the Atlantic (5). The distribution of precipitation by class amount shows that the fall season had a larger percentage of 1 to 10cm weekly precipitation periods than the other seasons (Table 1) for the 1984-1986 study period. As expected, winter had the greatest fraction of light precipitation periods.

Hydrogen

The pH of the wet deposition for the period of study does not show a trend over time (Figure 1). The overall average precipitation-weighted pH was 4.32 with the average varying by season as 4.33, 4.14, 4.46, and 4.35 for spring, summer, fall, and winter respectively. The concentration of H^+ was well correlated with $[SO_4^{2-}]$ over the entire study period (Table 2). This correlation between $[H^+]$ and $[SO_4^{2-}]$, as well as a fair correlation between $[H^+]$ to $[NO_3^-]$, was greatest during the summer and winter. Correlations between $[H^+]$ and $[SO_4^{2-}]$ and $[NO_3^-]$ were higher in this study than that found previously for West Lafayette, Indiana (3). Ammonium was also significantly correlated with $[H^+]$ during the summer and winter (Table 2), unlike that found for West Lafayette (3).

Table 1 Seasonal precipitation for Vincennes, Indiana for indicated years

	Weeks n	Weekly precipitation amount (%)						Deviation			1951-80 Normal (cm)
		<.5cm	0.5-<1cm	1.0-<2.5cm	2.5-<5cm	5.0-<10cm	>=10cm	1984	1985 (cm)	1986	
Spring	17	15.4	19.2	30.8	15.4	15.4	3.9		+6.04	-0.71	31.80
Summer	15	26.9	15.4	19.2	23.1	11.5	3.9		+1.22	+4.37	31.52
Fall	23	25.7	8.6	25.7	20.0	17.1	2.9	+15.90	+32.16	+3.92	22.61
Winter	14	34.5	17.2	13.8	20.7	13.8	0	+0.85	+2.16	-5.49	21.59

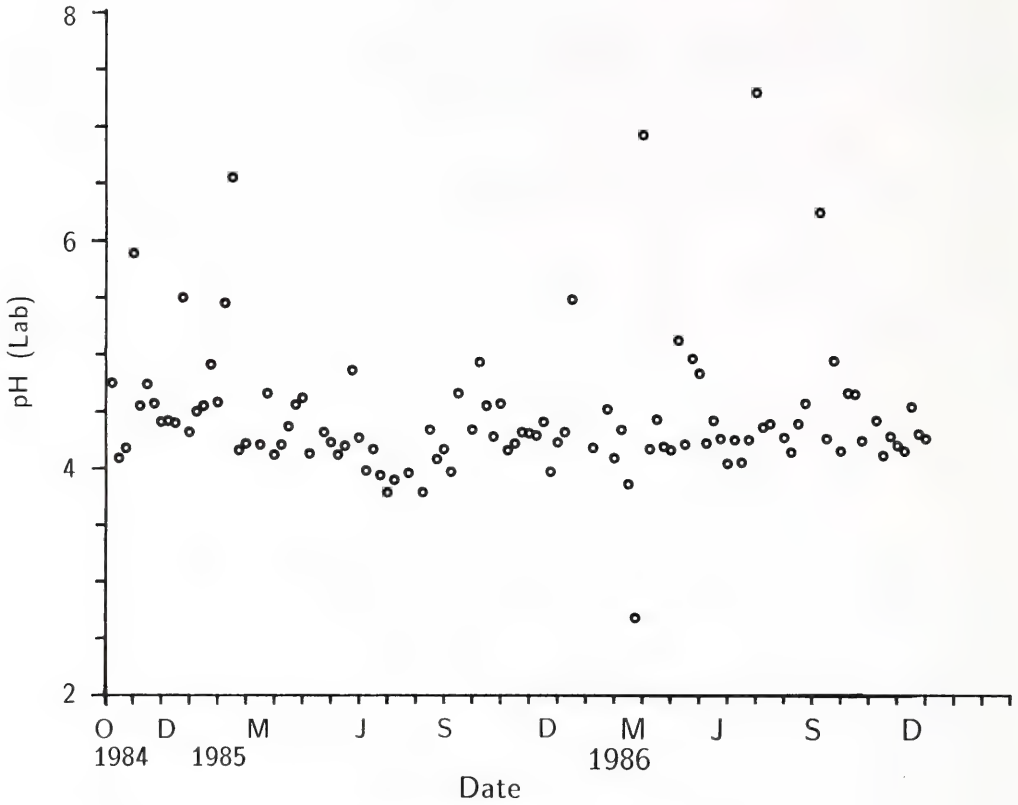

FIGURE 1. Variation in precipitation pH at Vincennes, IN.

The H^+ concentration was regressed (using the stepwise procedure) against all constituents. Over the entire study period, $[SO_4^{2-}]$ was associated with 62% of the $[H^+]$ variation while $[NO_3^-]$, $[NH_4^+]$, and $[Ca^{2+}]$ were assoicated with 11, 5, and 1% of the $[H^+]$ variation respectively. This is in contrast to the results of (3) that show $[SO_4^{2-}]$ associated with only 36% of the $[H^+]$ variation. Overall, the regression model accounted for 79% of the $[H^+]$ variation. Although the variables used in the regression model here differ from those used in the West Lafayette study (3), the total variance accounted for is the same.

Regression of H^+ concentration against all other precipitation constituents by season showed that the spring season NH_4^+ concentration was associated with 10% of the $[H^+]$ variation. The fall season regression was the only one in which $[NO_3^-]$ or any cation other than $[NH_4^+]$ was important in accounting for the precipitation H^+ concentration (Table 3).

The pH of the weekly precipitation was influenced by the amount of precipitation (Figure 2) with low precipitation corresponding to large variances in pH over the study period. Classifying the weekly precipitation, we find that the $[SO_4^{2-}]$ concentration was important in the estimation of H^+ concentration for precipitation in all categories except for the 0.5 to 1.0cm per week category where $[NO_3^-]$ was important in explaining the $[H^+]$ variance (Table 4). It is believed that the importance of the nitrate concentrations in accounting for the $[H^+]$ for this category of precipitation is probably an anomaly of the data set. While the scavenging of the particulates in the atmosphere is more efficient than that of gases (16), small weekly accumulations of precipitation do not show an expected greater importance in cations associated with soil dusts explaining the H^+ concentration.

The positive correlations between $[H^+]$ and $[NH_4^+]$ as well as those between

TABLE 2 Simple correlation matrix of precipitation constituent concentrations (All listed correlations significant at alpha = 0.05)

	NH_4	SO_4	NO_3	Cl	Ca	Na	K	Mg
H								
Spring		0.78	0.61					
Summer	0.89	0.94	0.90					0.81
Fall		0.52	0.39		−0.45		-0.52	
Winter	0.75	0.93	0.86		0.84		0.68	0.66
NH_4								
Spring		0.72	0.89		0.57			0.58
Summer		0.91	0.86		0.58			0.91
Fall		0.70	0.69	0.79	0.44		0.75	0.72
Winter		0.84	0.89		0.89		0.75	0.78
SO_4								
Spring			0.83	0.53	0.75	0.49		0.76
Summer			0.88					0.85
Fall			0.58					0.54
Winter			0.82		0.86		0.74	0.69
NO_3								
Spring					0.63			0.65
Summer					0.62	0.61		0.87
Fall								
Winter					0.97		0.81	0.87
Cl								
Spring					0.50	0.77	0.76	0.56
Summer							0.96	
Fall					0.56	0.60	0.67	0.76
Winter						0.83		
Ca								
Spring								0.99
Summer								
Fall							0.60	0.86
Winter							0.85	0.89
Na								
Spring							0.83	
Summer							0.94	
Fall							0.76	
Winter								
K								
Spring								
Summer								
Fall								0.42
Winter								0.73

$[H^+]$ and $[Ca^{2+}]$, and $[Mg^{2+}]$ are probably coincidental. This is supported by the negative coefficients in the majority of $[H^+]$ regressions both by season and precipitation amount.

TABLE 3 Association of H concentration variation for indicated constituents by season (alpha = 0.05)

Season	Partial regression r^2						Total R^2
	SO_4	NO_3	K	Mg	NH_4	Ca	
spring	0.60				(0.10)[1]		0.70
summer	0.89						0.89
fall	0.31	0.03	(0.27)	(0.32)			0.93
winter	0.86						0.86
OVERALL	0.62	0.11		0.05		0.01	0.79

1/parenthesis indicate negative regression coefficients

Sulfate

The weighted mean SO_4^{2-} concentration was greatest during the summer season and least during the fall and winter seasons (Table 5). Stepwise regression of $[SO_4^{2-}]$ on all other constituents resulted in the accounted for 84% of the over all $[SO_4^{2-}]$ variance using three independent variables—$[H^+]$, $[Mg^{2+}]$, and $[NH_4^+]$. The variation in H^+ concentration was associated with 62% of the variance in sulfate over the entire study period. Seasonal association of $[H^+]$ with $[SO_4^{2-}]$ variance was greatest during the summer and witner with 60, 89, 58, and 86% for the spring,

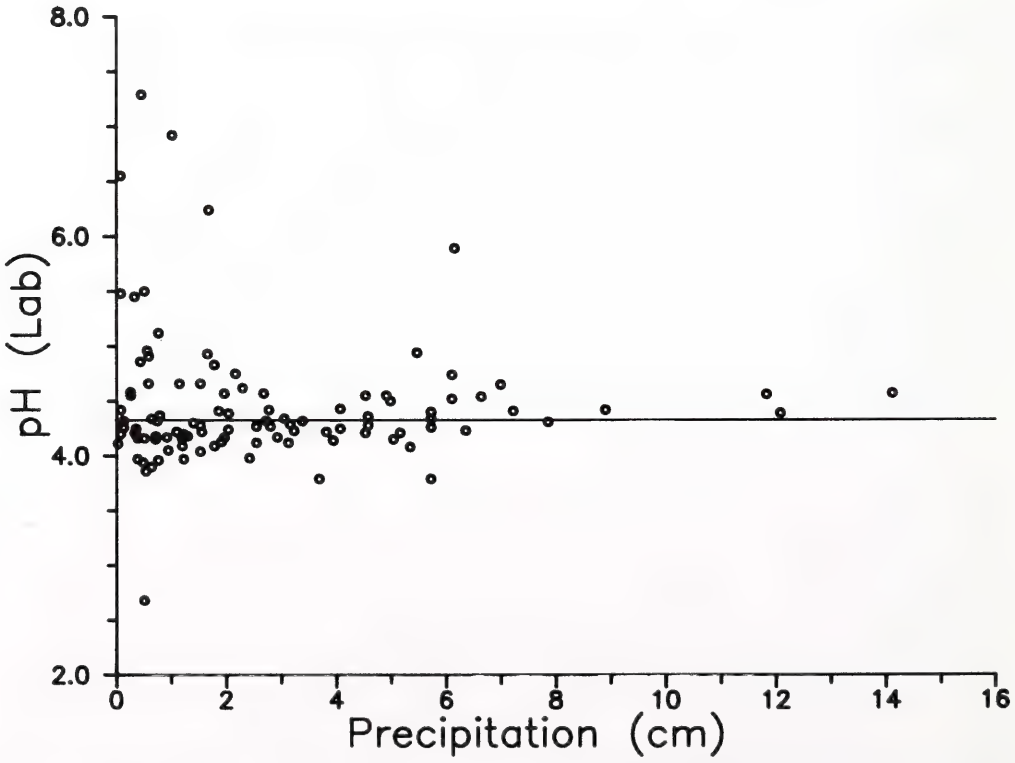

FIGURE 2. Effect of weekly precipitation amount on pH. Solid line designates the precipitation weighted mean pH.

TABLE 4 Association of H concentration variation with indicated constituent within precipitation amount (alpha = 0.05)

Weekly precip. (cm)	Partial regression r^2					Total
	SO_4	NO_3	NH_4	Ca	K	r^2
0.0-0.5	0.62					0.62
0.5-1.0		0.79	(0.14)[1]			0.93
1.0-2.5	0.63	0.08	0.04	(0.20)		0.89
2.5-5.0	0.92					0.92
5.0-10.0	0.69				(0.27)	0.96
<10.0			——	(only 3	samples)	——

1/parenthesis indicate negative coefficients

summer, fall, and winter seasons respectively. Homogeneous and heterogeneous oxidation of SO_2 by peroxides, oxygen, ozone, and hydroxyl compounds may explain the association of $[H^+]$ with the $[SO_4^{2-}]$ variance (8, 10). In general, SO_2 concentrations are greatest during winter period and lowest during summer period (1). Gas-phase oxidation of SO_2 has been estimated to be highest during the summer and lowest during the winter over St. Louis (7) while liquid-phase oxidation is approximately the same for all seasons (8, 14). The season of typically low SO_2 concentrations corresponds with a large fraction of the total annual precipitation, probably resulting in a proportionally higher liquid-phase versus gas-phase oxidation. The season of typically high SO_2 concentrations corresponds with a small fraction of the total annual precipitation (Table 1), probably resulting in proporationally higher gas-phase versus liquid-phase oxidation. This interplay of precipitation and gaseous concentrations may explain the apparent linkage of $[H^+]$ with $[SO_4^{2-}]$ during both summer and winter.

The magnesium concentration was associated with 6 and 30% of the SO_4^{2-} concentration variance in the spring and fall regressions respectively. Sea salt spray particulates of $MgSO_4$ compounds (2) and soil dust entrainment are the primary sources of $[Mg^{2+}]$. An indicator for determining the origin of the air mass depositing the sulfate is the sulfur to chloride (S/Cl) ratio. Maritime air consisting of sea salt spray typically has S/Cl ratios around 0.02 while continental air consiting of soil dust typically has S/Cl ratios of around 4. The average S/Cl ratio of the precipitation was 4.1,

TABLE 5 Selected seasonal anion and cation concentrations

	Ave. concentration[1] (mg/L)				
	SO_4	NO_3	Cl	NH_4	Ca
Spring	2.86	1.29	0.20	0.33	0.28
Summer	3.64	1.52	0.39	0.23	0.23
Fall	2.34	0.84	0.24	0.17	0.27
Winter	2.34	1.21	0.25	0.20	0.10

Note: 1. precipitation weighted average

3.4, 2.9, and 2.9 for spring, summer, fall, and winter respectively. These high S/Cl ratios suggests a continental source for much of the $[SO_4^{2-}]$.

Ammonium explained 24 and 5% of the variance in $[SO_4^{2-}]$ for the spring and winter seasons respectively. The primary sources of NH_4^+ are naturally occurring products of decomposition. Ammonium sulfate is one of the components present in the water soluble part of fine particulate aerosols (4).

Nitrate

The weighted mean NO_3^- concentration was greater during the summer and least during the fall season (Table 5). Stepwise regression of all constituents on $[NO_3^-]$ resulted in the association of 73% of its variance with $[H^+]$, $[NH_4^+]$, and $[Ca^{2+}]$.

Hydrogen concentration was associated with 12 and 14% of the $[NO_3^-]$ variance in the spring and fall respectively and 80% in the summer season. One likely source of the association of $[H^+]$ with $[NO_3^-]$ in the summer is the gas-phase oxidation of NO_x by hydroxyl compounds and hydrocarbons (7). The primary product of these reactions is nitric acid vapor. In contrast to SO_2 oxidation, liquid-phase oxidation does not appear to be significant within the atmosphere (7). Overall, the seasonal differences in NO_x oxidation, both gas and liquid-phase, is poorly understood.

Ammonium concentration was associated with 56 and 47% of the $[NO_3^-]$ variation in the regressions for spring and fall respectively. Ammonium nitrate (NH_4NO_3) is a common form of argicultural fertilizer applied in the spring and fall and as such is a potential cause for this apparent linkage in precipitation chemistry. As previously mentioned, natural decomposition is the primary source of naturally occurring NH_4^+. Some evidence exists to suggest that gaseous NH_3, gaseous HNO_3, and soild NH_4NO_3 have a temperature sensitive equilibrium in the atmosphere which may provide the basis for this association (10, 15).

Calcium was associated with 3 and 10% the $[NO_3^-]$ variance during the spring and summer. Calcium concentration in the precipitation was least during the winter (Table 5) yet was associated with 93% of the $[NO_3^-]$ variance. Apparently the overall particle-acid chemistry in the winter is different from that of the other seasons.

Chloride

The weighted mean Cl^- concentration was greatest during the summer season and least during the spring season (Table 5). Stepwise regression of all constituents on $[Cl^-]$ resulted in the association of 82% of the variance with $[Na^+]$, $[Mg^{2+}]$ and $[Ca^{2+}]$.

Sodium was associated with 67% of the $[Cl^-]$ variance for the overall study period. By season, $[Na^+]$ was associated with 93, 8, 69, and 59% of the $[Cl^-]$ variance in the spring, summer, fall, and winter respectively. The high association of $[Na^+]$ with $[Cl^-]$ variance in the spring and fall corresponds with the high association of $[Mg^{2+}]$ and $[SO_4^{2-}]$ reported above. The high associations may be a result of the prevalence of Na^+ and Mg^{2+} in soil dust aerosols as well as salt sea spray. While road salt is another potential source of Na^+ in conjunction with Cl^-, the high associations do not occur during periods of road salt use and road salt is not used in the vicinity of the collector. Furthermore the sodium to chloride ratio (Na/Cl) can be used to differentiate road salt, sea salt spray, and soil dust. The expected sea salt ratio of Na/Cl is 0.56 (the solid line of figure 3). Studies of sea salt spray however show that the Na/Cl ratio is actually closer to 0.37 above the ocean (9). The Na/Cl ratio of road salt is 1.0 (the dotted line of figure 3) while that for soil dust is greater than 1.0. The average Na/Cl ratio for each season was

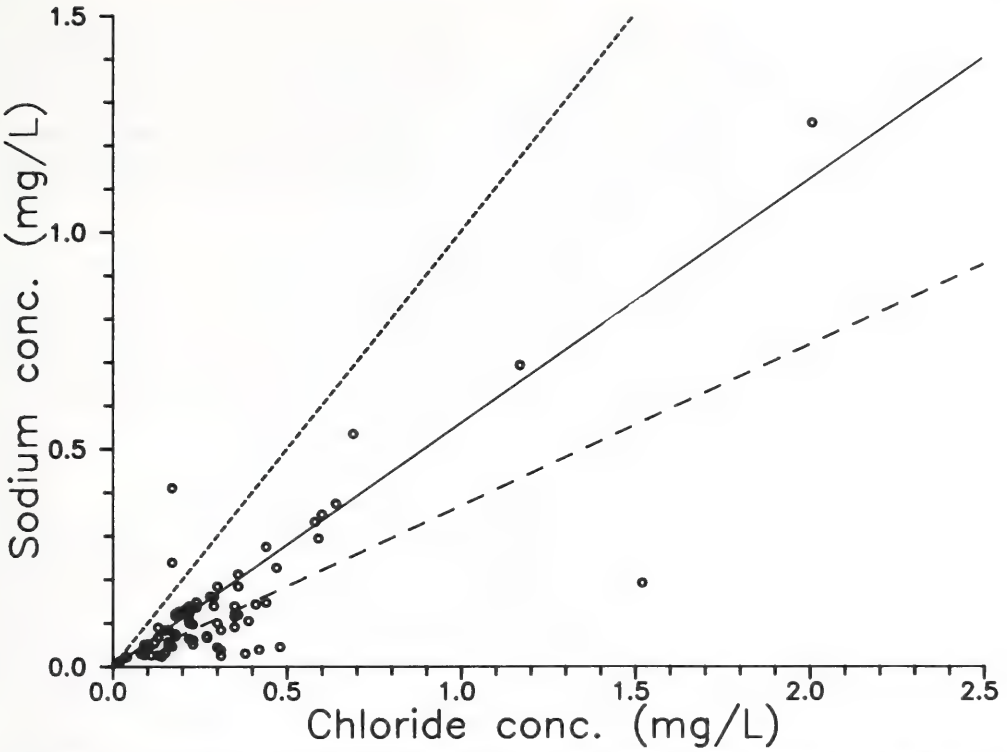

Figure 3. Sodium - chloride concentration relationships for weekly precipitation. The sea salt Na/Cl ratio of 0.56 is indicated by solid line. The sea salt spray Na/Cl ratio of 0.36 is indicated by the dashed line. The road salt Na/Cl ratio of 1 is indicated by the dotted line.

0.41, 0.40, 0.46, and 0.49 for spring, summer, fall and winter respectively—close to that expected from sea salt.

Magnesium was associated with 11% of winter [Cl^-] variation. A similar evaluation of the chemical components in association with the [Mg^{2+}] variance showed [Ca^{2+}] to be the dominant association during the winter. Calcium and [K^+] was associated with 3 and 2% of the overall Cl^- concentration variation respectively. These associations may be related to soil dust, fly ash, or sea salt spray (9). Seasonally, [K^+] was present in the stepwise regression in summer and winter while [Ca^{2+}] was not found in the final regression for any season. It is suspected that the variance in these metals is fortuitous as negligible [Cl^-] is present in flyash or soil dust (15).

Hydrogen concentration was associated with 13 and 18% of the fall and winter [Cl^-] variation. The explanation of [Cl^-] variation by [H^+] in the fall is probably not a result of gas-phase chlorine oxidation since the regression coefficient was negative. The winter partial r^2 may be indicative of oxidation reactions producing hydrochloric acid. Hydrochloric acid is one of the components present in the water soluble part of fine particulate aerosols (4), originating largely from lead-based automobile exhausts.

Ammonium

The weighted mean [NH_4^+] was greatest during the spring season and least during the fall (Table 5). Ammonium tends to increase the pH of precipitation by reacting with SO_4^{2-} to form salts under conditions of high NH_3 gas concentrations (4). Livestock waste and other organic matter decomposition are probably sources of most of the NH_4^+, but some NH_4^+ is also found in sea salt spray. The variation in [H^+] associated with [NH_4^+] is particularly important during the fall.

Calcium

The weighted mean Ca^{2+} concentration was greatest during the spring and fall seasons and least during the winter (Table 5). The relatively high correlations of $[Ca^{2+}]$ with $[SO_4^{2-}]$ and $[NO_3^-]$ support the contention by (3) that the calcium is in the form of more neutral salts for all seasons but the fall (Table 2). It is generally assumed that the calcium found in precipitation is from soil dust entrained in the atmosphere or flyash (9). However an additional source is maritime sea salt (16). The dominance of a soil dust source is suggested by those results as flyash should be present heavily in summer and winter and less so in fall and spring as heating and cooling needs are slack. During the fall, the sea salt may be a significant contributor of $CaCO_3$. This however is unlikely since the mean cation to anion ratio for the fall season was 0.98 indicating some excess anions rather than excess cations in the precipitation.

1985 Fall season anomaly

Fall is unique in the data set since the 1985 precipitation greatly exceeded the normal (Table 1), and the excess precipitation was largely associated with tropical storms and hurricanes (5). Precipitation from these storms resulted in rainout and washout of atmospheric gases and particulates largely originated from maritime regions. The fall of 1984 also has high precipitation but was derived from more typical midlatitude cyclones (6) with the precipitation causing rainout and washout of atmospheric gases and particulates advected from both maritime and continental regions.

The fall seasons of 1984 through 1986 had unusual positive correlations between hydrogen and the other cations (Table 3, 4). The importance of the cations is evident in the fall where the combined $[Mg^{2+}]$ and $[K^+]$ were associated with 33% of the $[SO_4^{2-}]$ and 59% of the $[H^+]$ variance in the precipitation samples. During this season, the pH is highest. For the 1984 and 1986 fall seasons ($n = 15$ weeks), the expected negative correlations between $[H^+]$ and the variance cations appear (except $[NH_4^+]$) and correlations between $[H^+]$ and $[SO_4^{2-}]$, $[NO_3^-]$, and $[NH_4^+]$ improve. Only 25% of the H^+ concentration variance in the 1984 and 1986 fall seasons was associated with $[Na^+]$ and $[Ca^{2+}]$. For the 1984 and 1986 fall seasons, 32% of the SO_4^{2-} concentration variance was associated with cations ($[Mg^{2+}]$ and $[Na^+]$). For the 1985 fall season ($n = 7$ weeks), only $[NH_4^+]$ was significantly associated with $[H^+]$ variance, with only 69% of the $[H^+]$ variance accounted for. The 1985 fall season $[SO_4^{2-}]$ variance was primarily explained by $[Ca^{2+}]$ (92%) with only 7% explained by the H^+ concentration. The importance of $[Ca^{2+}]$ in the 1985 fall regression may be due to $[Ca^{2+}]$ present in maritime air sea salt particulates carried by the tropical storms of the season.

NH_4^+ concentrations accounted for most of the $[Cl^-]$ and $[NO_3^-]$ variance during the combined 3-year fall season while $[SO_4^{2-}]$ and $[Mg^{2+}]$ accounted for 90% of the $[NH_4^+]$ variance. Removing the 1985 fall data results in a reduction of explained $[NH_4^+]$ variance to 48%—all associated with $[NO_3^-]$. Looking exclusively at the 1985 fall samples, 99% of the $[NH_4^+]$ variance and 88% of the $[NO_3^-]$ variance is associated with the Cl^- concentration. This dominance of $[Cl^-]$ in the variance of $[NH_4^+]$ and $[NO_3^-]$ for the 1985 fall season may be due to the reaction of sea salt spray particulates carried in the storms with NH_3, HNO_3, H_2SO_4 and SO_2 resulting in the replacement of Cl^- by NO_3^- and SO_4^{2-} as seen in a remote maritime atmosphere (2).

Comparing the 1985 fall to the 1986 and 1984 fall season samples with one-tailed t-tests show significant differences between $[SO_4^{2-}]$ and $[H^+]$ (at 0.10 level) and in the Na/Cl ratio, the S/Cl ratio and the Cl concentration (at 0.15, 0.20,

and 0.20 level respectively). The 1985 season had higher concentrations of H^+, Cl^-, and SO_4^{2-} and lower Na/Cl and S/Cl ratios than the 1984 and 1986 combined fall seasons. The average 1985 Na/Cl ratio was 0.39 which is very close to the predicted ratio of sea salt spray, while the average 1984 and 1986 Na/Cl ratio was 0.63 indicating more continental influence on the precipitation chemistry.

Summary and Conclusions

The chemical composition of the precipitation at Vincennes, Indiana collector over the period of October 1984 through December 1986 appears to be of both natural and man-made origin. The variance in the precipitation pH was greater for weekly samples in which only 2cm of precipitation was collected than for samples of greater than 2cm of precipitation. Seasonally, the precipitation pH was lowest during the summer and highest during the fall with an overall precipitation weighted average pH of 4.32.

The oxidation reactions of SO_2 (resulting in SO_4^{2-}) appear to the most important factor in explaining the acidity of the precipitation for all seasons except fall. The oxidation reactions of NO_x (resulting in NO_3^-) however appear to contribute little to the precipitation acidity, but do appear to be more active during the summer than the rest of the year. The nitrate present in the precipitation is closely linked with the ammonium in the spring and fall suggesting that fertilizers and decomposition of natural materials are large contributor to the nitrate. The high correlations between $[SO_4^{2-}]$ and $[NO_3^-]$ existing throughout the seasons cannot be explained readily but is frequently observed. Based on the regressions, the precursors of SO_4^{2-} formation differ significantly from those of NO_3^- formation. This however must be tempered by the fact that associated variance of a given constituent to SO_4^{2-} or NO_3^- does not a priori mean chemical interaction.

The series of recurring major tropical storms in the fall of 1985 appeared to significantly alter the precipitation chemistry. Compared to the fall season of 1984 and 1986, the fall of 1985 had reduced pH and elevated SO_4^{2-} and Cl^- concentrations, an apparent reduced importance of oxidation reactions for SO_4^{2-} formation, and an increased importance of $[Ca^{2+}]$, $[Cl^-]$, and $[NH_4^+]$ in the precipitation chemistry.

Calcium, Mg^{2+}, and K^+ are probably all primarily present as a result of soil dust entrainment. Evaluation of the 1985 fall season anomalous chemical relationships suggest an interaction of these cations with maritime air mass particulates. Excluding the 1985 fall season, the cations were still associated with 35% of the $[H^+]$ variance including this fall season to be chemically unique within the period of study.

In comparison to a study of the wet deposition at West Lafayette, IN for an earlier period of time (3), the Vincennes site acidity ($[H^+]$) appears to be much more associated with $[SO_4^{2-}]$ than at the West Lafayette, IN site. However regression of $[H^+]$ against all the chemical constiuents for both Vincennes and West Lafayette showed the same fraction of the $[H^+]$ variance accountable by the ions studied.

Future studies evaluating the trends in precipitation chemistry should take into account inter-annual variability in the storm tracks and the intensity and duration of the storms producing the weekly precipitation.

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ZOOLOGY

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ABSTRACTS

Effects of Bee Pollen as the Sole Source of Nutrition on Survival of Laboratory Mice. Timothy J. Burkholder and Valarie J. Ennis, Department of Biology, Taylor University, Upland, Indiana 46989.——It has been proclaimed that bee pollen is the most complete food man has ever discovered. Some estimates indicate that tons of bee-collected pollen are processed annually and a significant portion is being used as a supplement food stuff for humans. Experimental animal studies in this country concerned with the physiological and pharmacological effects of bee pollen are almost non-existent.

Inbred laboratory mice of the ICR strain were placed adlibitum at twenty-one days of age on distilled water and three different forms of commercial bee pollen as the sole source of food intake. Many of the mice have survived in a healthy condition studied for periods of over one year.

It was not possible to determine whether the success or failure of the different forms of bee pollen was related to differences in nutritional value of each. Further studies are in progress and others are being planned for implementation in the near future.

Evaluation of Endangered Fragile Heelsplitter Mussels (*Potamilus ohiensis*) Using Species/Time Visual Census Methods: Blue River, Southern Indiana, Ohio River Drainage. William D. Ehringer, Claude D. Baker, and Bill J. Forsyth, Department of Biology, Indiana University Southeast, New Albany, Indiana 47150.——We evaluated the status of the endangered fragile heelsplitter (*Potamilus ohiensis*) using two species/time visual census methods (Rapid Visual Census—RVC and Visual Fast Count—VFC). Based on comparison with 5,000 shells collected at three sites, the VFC was found to provide a quick, reliable characterization of the mussel fauna. Of 25 species, fragile heelsplitters and eight other mussels were taken in very low numbers. However, the finding of eight fresh fragile heelsplitter shells suggests that this species is surviving in low numbers in the lower Blue River.

The Interrelationship between the Renin-angiotensin System and Catecholamines in the Trunk Perfused Rainbow Trout. R.J. Ferlic, Department of Biological Sciences K.R. Olson, SBCME; University of Notre Dame, Notre Dame, Indiana 46556 and D.W. Duff, Department of Biology, Indiana University at South Bend, South Bend, Indiana 46634.——Angiotensins I and II (AI and AII) and catecholamines (CAs) have profound effects on blood pressure regulation in bony fish; when injected *in vivo*, both increase blood pressure. Questions arise regarding the interaction of these two pressor substances and their sites (ie. vascular beds) of action. A dual perfused

trunk preparation in which the coeliacomesenteric artery (CM: perfusing the viscera) and dorsal aorta (DA: perfusing the kidney and skeletal muscle) were perfused separately was used to examine these questions. The two vascular beds responded differently to angiotensins: AI and AII were equipotent in increasing DA vascular resistance (VR) whereas only AII increases VR in the CM and then only at higher concentrations (10-6M). AI effects in the DA could be blocked by the angiotensin coverting enzyme inhibitor captopril. The alpha-adrenergic blocker, phentolamine, inhibited the DA response to AII by 40% indicating that a portion of the response to AII is mediated by catecholmines. Phentolmaine did not affect the response of CM to AII. Supported in part by NSF:PCM84-04897.

Population Estimates of Spotted Darters (*Etheostoma maculatum*) and Bluebreast Darters (*Etheostoma camurum*) in the Lower Blue River, a Southern Indiana Tributary of the Ohio River. Terry E. Griffis, Claude D. Baker, Bill J. Forsyth, Department of Biology, Indiana University Southeast, New Albany, Indiana 47150.——We used a repeated stripping or Zippin method to evaluate spotted and bluebreast darter population in the Blue River. A total of 130 fishes were taken at four downstream sites. Of these, some 26 bluebreast darters and nine spotted darters were taken. Extrapolation to numbers per seven mile section of primary habitat yielded working ballpark estimates of 15,000 bluebreast darters and about 4,500 spotted darters. These data are to be regarded as working estimates which can be refined as we continue the work of mapping riffles and generating data from additional sites.

Evidence for a Kallikrein-kinin System (KKS) in Rainbow Trout (*Salmo gairdneri*). David W. Lipke and Kenneth R. Olson. SBCME, Indiana University School of Medicine, Notre Dame, Indiana 46556.——Few studies have examined the presence of a KKS in anamniotic vertebrates, though some components of this system are present in amphibians and fish. Our laboratory has been conducting research to identify a KKS in trout and we have now compiled evidence that these fish posses all functional components of a mammalian-like KKS. We determined that; 1) kallikrein-like activity was observed in both trout gills and kidney using three different assays for kallikrein, 2) bradykinin (BK) administered intra-arterially, caused dose-dependent increases in systemic blood pressure (BP) of chronically cannulated trout (CCT), 3) glandular kallikrein (GK) increased BP of CCT pre-treated with the angiotensin-converting enzyme inhibitor, captopril, 4) GK liberated a substance (T60K) from trout plasma *in vitro* that increased arterial BP of CCT and catheterized rats, 5) the isolated, perfused trout gill selectively extracted ^{3}H-BK from perfusate traversing the respiratory circulation, 6) homogenates of gill tissue were observed to metabolize BK and these tissue homogenates also inactivated T60K. This evidence supports the proposal that a KKS is present in salmonid gills.

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Imidazo (1,2- α) pyridine Therapy of Enteral Trichinellosis. Richard O. McCracken and Elizabeth M. F. Merkli, Department of Biology, Indiana University-Purdue University at Indianapolis, Indianapolis, Indiana 46223.—Mice experimentally infected with the parasite *Trichinella spiralis* were used to test the therapeutic effectiveness of a new anthelmintic, methyl 6-(phenysulfinyl) imidazo (1,2- α) pyridine-2-carbamate, against the adult worms during the intestinal phase of infection. A single oral dose of 100 mg/kg of the drug on the third day after exposure to infection was totally ineffective against the adult worms as determined at necropsy on day 6. Furthermore, neither division of the daily oral dose nor increasing the length of the treat-

ment period from 1 to 4 days enhanced drug activity *in vivo*. In summary, the drug methyl 6-(phenylsulfinyl) imidazo (1,2-α) pyridine-2-carbamate was totally inactive against adult *Trichinella spiralis* at any of the dosages tested in this study.

Is the Meningeal Worm, *Parelaphostrongylus tenuis* (Nematoda: Metastrongyloidea), Present in White-tailed Deer in Indiana? THOMAS R. PLATT, Department of Biology, Saint Mary's College, Notre Dame, Indiana 46556.—*Parelaphostrongylus tenuis*, a common parasite of white-tailed deer (*Odocoileus virginianus*) in eastern North America, has not been reported from Indiana. Fresh fecal samples were collected from 62 hunter-killed deer during the 1986 hunting season from the Kingsbury Fish and Wildlife Area, Laporte County. Feces were examined using a modified Baermann technique. Twenty nine samples (47%) were positive for larvae identical to those of *P. tenuis*. All age classes were infected. The number of larvae per gram of feces ranged from 0.2 to 498. Identification of *P. tenuis* was confirmed by finding the infective stage (L3) in field collected mollusks from Kingsbury during the summer of 1987. Three hundred and seventy one slugs and snails, representing 14 species, were examined by artificial digestion or pressing the animal between glass slides. *Cochlicopa* sp. (1/18), *Deroceras laeve* (1/32), and *Discus cronkhitei* (2/189) were positive for *P. tenuis* larvae. Deer have not been examined for the presence of the adult worm and the presence of related metastrongyloids at Kingsbury cannot be discounted.

Skeletal Ossification as an Indicator of Teratogenic Exposure. CHARISSA URBANO AND MONICA NORDENBROCK, Department of Biology, Ball State University, Muncie, Indiana 47306.—The toxic effects of drugs on fetal development may manifest themselves not only in terms of malformations but also in terms of retarded fetal development. Following exposure of the dam to acetazolamide and/or caffeine fetal C57BL/6J mice were stained with Alizarian red in preparation for skeletal examination of ossification centers in cervical and caudal vertebrae. At all teratogenic doses there was a significant decrease in the frequency of ossified caudal vertebrae. Cervical vertebrae also provided a sensitive index of teratogenic exposure with the first cervical vertebra (C1) being most often affected.

Cytogenetics of Coatimundis from the Potawatomi Zoo. DAWN M. VERLEYE, CHRISTINE DIDONATO AND THOMAS A. FOGLE. Department of Biology, Saint Mary's College, Notre Dame, Indiana 46556.—A G-banding analysis was conducted of *Nasua nasua* and *Nasua narica* to search for cytogenetic differences among members of a zoo colony. Peripheral lymphocytes were drawn by venipuncture and cultured for 72 hours in three different media to optimize cell growth. Karyotypes from five females revealed hybridization resulting from complex chromosome rearrangements. There are no previous reports of banding analysis or cytogenetic variability in either species. Application of the results from this study will be useful for improving breeding management and further characterization of the genus.

Technique for Determining Nesting Success in the American Goldfinch. DORIS J. WATT AND AMY M. DIMBERIO, Department of Biology, Saint Mary's College, Notre Dame, Indiana 46556.—We examined 21 nests of American Goldfinches collected in December 1986. Six qualitative characters (presence of excrement, feather sheaths, eggshells, insect remains, thistle down and nest lining) and nine quantitative measures (widest nest diameter, narrowest nest diameter, inner nest depth, total nest depth, circumference at rim, widest cup circumference, thickest rim thickness, thinnest rim thickness and

cup volume) were analyzed. Based on known success/failure of six of the nests, we determined that the presence of feather sheaths in the nest was the best predictor of nesting success.

A Morphological and Behavioral Description of Cercaria *Sanguinicola sp.* Scott P. Wiley and David L. Daniell, Department of Biological Sciences, Butler University, Indianapolis, Indiana 46208.—An Apharyngeate Lophocercous cercaria which develops in *Pseudosuccinea columella* was discovered in lakes of Northern Indiana and Southern Michigan. It apparently is a new species belonging to the family Sanguinicolidae (blood flukes of fish). Morphological and behavioral aspects of the cercaria were studied over a 10 week period, and its morphology was compared with those of previously described *Sanguinicola* species. Measurements in micrometers of body, tail stem and furca averaged 68.1 μm x 22.7, 267.8 x 20.4, and 65.8 x 9.0 respectively. Incidence of infection varied from two percent in June to 30 percent in early August. Fish collected from lakes, as well as laboratory exposed fish were examined in an attempt to isolate adult worms and identify the definitive host.

Insect Pathology—What's Happening? Harold L. Zimmack, Department of Biology, Ball State University, Muncie, Indiana 47306.—In 1962, Rachel Carson wrote *The Silent Spring*, and alerted us to the indiscriminate and excessive use of pesticides. If insecticides killed insects they could also kill man and animals. DDT and non-biodegradable hydrocarbons were banned throughout the world. Steinhaus had provided a strong foundation in insect pathology that could enable scientists to select microorganisms as biological control agents against destructive insects. It was a "golden opportunity" for insect pathologists. Insect pathologists include specialists in virology, bacteriology, mycology or microsporidia but a coordinated team-effort is required to successfully select effective insect pathogens.

If man is infected by more than 100 disease organisms, there must be many microorganisms that can cause disease in any given destructive insect. Possible approaches to addressing this problem will be proposed.

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Studies of the Habitat and Food of Sympatric Populations of *Plethodon cinereus* (Green) and *Plethodon dorsalis* Cope in South Central Parke County, Indiana

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Introduction

The purpose of this study was to examine the ecological relationships between sympatric populations of the closely related red-backed salamander, *Plethodon cinereus* (Green), and the zigzag salamander, *Plethodon dorsalis* Cope. At one time regarded as conspecific, these salamanders are now established as separate species (Thurow, 1955, 1956; Highton, 1962).

Highton (1962) stated that sympatric populations of the two species occur in Georgia, Tennessee, Oklahoma, and Indiana, and that hybridization had not been reported. Highton (1962) also said that the two species appear ecologically separated even when occurring sympatrically, with *P. cinereus* being associated with the forest floor and *P. dorsalis* being associated with ravines and exposed rock. Rubin (1967) found no gross differences in the habitats of the species but noted that they were rarely found under the same object.

The objectives of this study were to compare the habitats (soil moisture and cover) and food of these two species in a Parke County, Indiana area where both occur.

Description of the Study Area

The study area was located in south central Parke County, Indiana, one and a half miles north of Rosedale and one mile east of Coxville, on a west-facing slope above the Raccoon creek flood plain. In many places the slope is cut by ravines, often the result of coal mining which took place around 1920. Outcroppings of limestone overlying sandstone are frequent along the slope. The soil ranges from forest humus in depressions and gently sloping areas to sandy gravel in steeper areas. A major portion of the study area was below a large limestone cliff.

The forest is beech-maple along the slopes and ravines, grading to oak-hickory in drier situations. Much of the forest is old growth, and in places there is undergrowth of sassafras (*Sassafras albidum*), pawpaw (*Asimina triloba*), redbud (*Cercis canadensis*) and other shrubs. Herbaceous plants, particularly touch-me-not (*Impatiens* sp.), are often dense in less shaded areas. Ferns are common in the ravines.

Methods and Materials

Salamander studies are often based on specimens collected by sampling methods that could introduce bias, such as by collecting only under stones and logs and by disregarding leaf litter. In order to eliminate this bias, all cover was examined in randomly selected plots.

This study was based on 71 individuals of *P. dorsalis* and 72 of *P. cinereus* taken between September 24 and November 22, 1967, from random plots within the study area. The salamanders were preserved in alcohol.

Salamanders were later examined with a dissecting microscope and measured with calipers and a millimeter ruler. Species, pattern, secondary sex characteristics,

and snout-vent length were noted. The stomach was removed and its contents identified. The intestine was examined for parasites. The gonads were measured, and color and degree of development were noted. In each mature female, the number of developing eggs was counted, and the largest and smallest eggs were measured.

The study area was a 1000 by 2000 foot wide area along an east-west slope parallel to the ridge. This area was mapped, divided into wet (stratum I) and dry (stratum II) portions, and divided into 8000 five by five foot plots, which were numbered consecutively. Random plots for study were selected via a random numbers table. Plots were located in the field by pacing appropriate distances from the nearest permanent marker to the northwest corner of the plot.

The moist stratum consisted of 1194 plots lying below a limestone cliff and along ravines. Stratum II consisted of the remainder of the area. A total of 266 plots was examined from August 27 to November 22, 1967. A total of 221 plots was examined in the moist area, with 77 yielding 143 salamanders, whereas the 45 plots in stratum II yielded three salamanders. A border of leaf litter six inches wide surrounding each plot was removed so that any organisms moving to or from the plot could be seen. A sketch was made showing rocks, woody material and leaf litter. Percentage estimates were made of the amount of canopy cover, understory cover and herbaceous cover as each of these appeared looking down upon the plot. The per cent of the individual species forming each cover layer was noted. An estimate of per cent leaf litter, woody material and stone covering the surface of each plot was made. Leaf litter depth was estimated. Soil texture (humus, sand, gravel or clay) and per cent slope were recorded. A soil sample was placed in a plastic bag for later moisture estimates. Soil samples were weighed, then dried in a drying room at approximately 40 °C and reweighed until no further weight loss occurred for two consecutive weighings. Soil moisture was recorded as per cent wet weight.

Each plot was examined for salamanders and invertebrates by removing all movable rocks and wood material and sorting through all leaf litter and debris. At the time of capture, general characteristics of each salamander (species, size, color phase, and pattern irregularities) were recorded. One square foot of leaf litter around each salamander was collected and placed in a plastic bag for examination for invertebrates. The cover the salamander was under was noted.

Leaf litter samples were placed in Berlese funnels until dry to extract invertebrates. Invertebrates were preserved in 70% alcohol and identified and counted with the aid of a dissecting microscope.

Results

Size Distribution

Snout-vent lengths of the salamanders were graphed (Figure 1). They fell into three groups. These groups probably reflect the ages of the salamanders, the first group representing hatchlings of the year (13-22 mm.), the second representing salamanders one year old (23-32 mm.), and the third representing salamanders two or more years old (33-47 mm.). This assumption is supported by the fact that the salamanders in the third group were mature individuals and Thurow (1955) indicated that both species mature in two years. These groups form the three size classes referred to throughout this paper.

The snout-vent length distributions of the two species indicate that their maturation rates are similar. Both species mature in two years, and growth is slower after maturation.

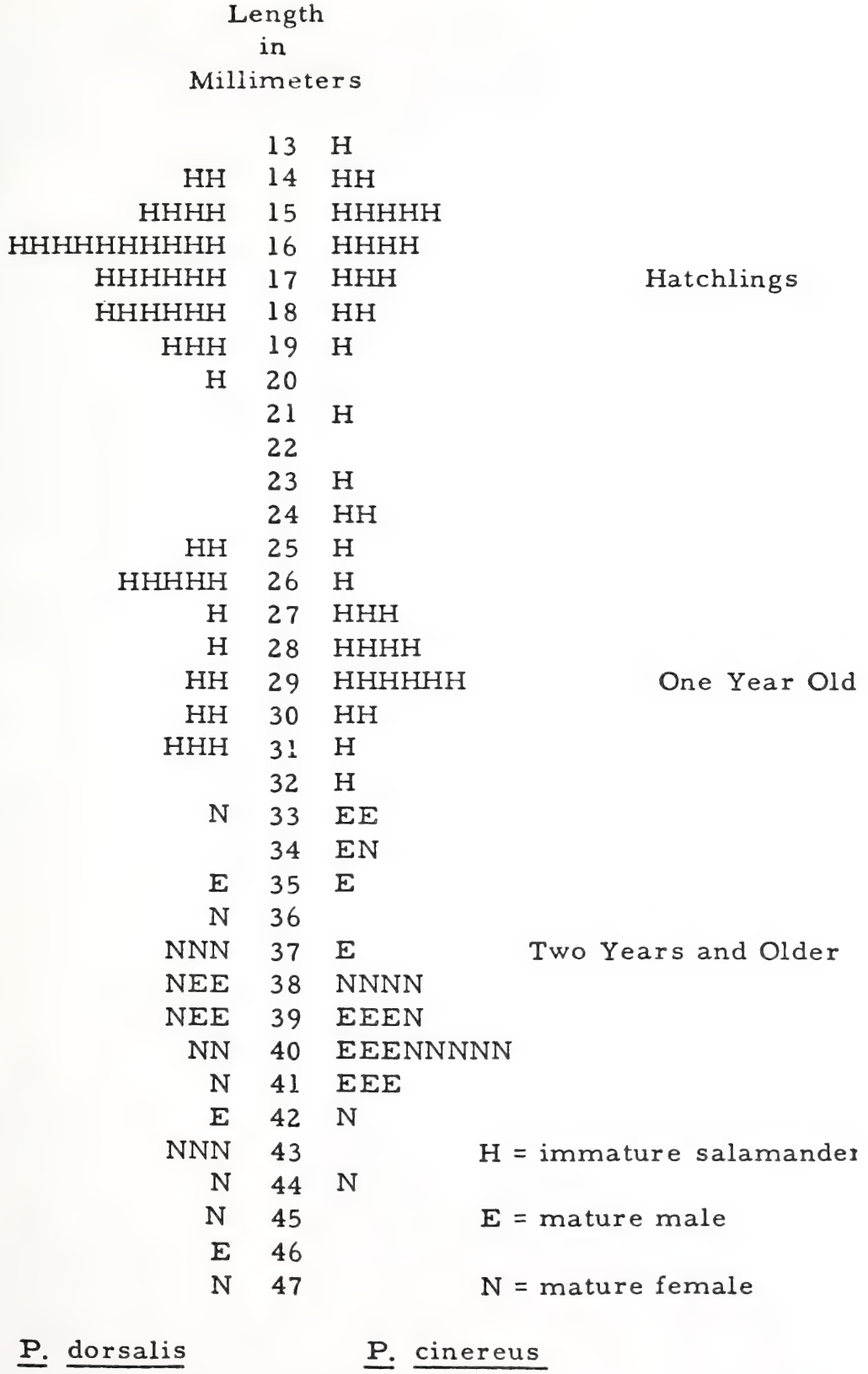

FIGURE 1. Snout-vent lengths of 71 *P. dorsalis* and 70 *P. cinereus* taken in south central Parke County, Indiana, during the fall of 1967.

Sex Ratios

During the study 28 mature individuals of *P. cinereus* were taken, 14 males and 14 females, twenty-three of *P. dorsalis*, seven males and 16 females. Although this difference contrasted markedly with *P. cinereus* and its 50:50 ratio, it was not significant (Chi-Square = 1.76, 1 df), and may have been due to chance. However, such sex ratios have been reported for other Plethodons (Dumas, 1956).

Eggs

The mean number of developing eggs in 15 mature individuals of *P. dorsalis* was 6.33 and in 14 of *P. cinereus* it was 8.29. This difference was not significant (T = 1.703, 27 df). These means were slightly higher than means reported by Thurow (1955) (*P. dorsalis*, 4; *P. cinereus*, 7).

Cover

Highton (1962) reported that the two species appeared ecologically separated in sympatric populations with *P. cinereus* being associated with the forest floor and *P. dorsalis* with exposed rock and ravines. Comparison of the average cover percentages per plot of the three major types of cover (leaf litter, rock and wood) revealed no significant differences between the cover types utilized by these species (Table 1).

TABLE 1. Occurrence of salamanders by size class and per cent per plot of different cover types for 68 *P. dorsalis* and 71 *P. cinereus* from Parke County, Indiana, during the fall of 1967

	P. dorsalis			*P. cinereus*		
Size Class	Wood	Rock	Leaf Litter	Wood	Rock	Leaf Litter
10-24 mm.	8	10	12	2	4	11
25-34 mm.	5	7	5	3	13	13
35+ mm.	4	16	11	6	8	11
Total	17	23	28	11	25	35
Avg. % cover per plot	5.0	23.5	64.5	5.5	23.9	66.5
No. salamanders expected from avg. % cover	3.7	17.2	47.2	3.9	17.6	49.2
% salamanders occurring in cover types	24.3	32.9	40.0	15.5	35.2	49.3

Both were in significantly greater numbers under wood and rocks and fewer under leaf litter (*P. cinereus*, Chi-Square = 19.91, 2 df; *P. dorsalis*, Chi-Square = 54.2, 2 df). There was no significant difference in cover between *P. dorsalis* and *P. cinereus* (rock, Chi-Square = 0.002, 1 df; leaf litter, Chi-Square = 2.61, 1 df; wood, Chi-Square = 0.79, 1 df) but the figures suggest that *P. cinereus* may have a slight preference for leaf litter and *P. dorsalis* for wood cover, while both have nearly equal affinity for rock (Table 1).

There was no apparent correlation between size class and cover either within or between species (Table 1).

The two species were found occurring together in 16 of the 221 plots examined in the favorable strata. This is significantly greater than would be expected by chance (Chi-Square = 108.1, 1 df).

Rubin (1967) reported that the two were seldom found under the same object. This was true in the present study, except for two instances. Two immature individuals were found under a rock and two mature females were found in a depression under leaf litter.

Moisture

Comparison of the per cent moisture at the site of collection showed a significant difference between the two species (Table 2). *P. dorsalis* was found in the moister habitat ($F = 5.53$, $df = 1,137$). Comparison of the moisture of plots containing

TABLE 2. Average per cent moisture of plots.

Type of Plot	Number of plots examined	Number of salamanders captured	Average % moisture
Stratum I (less favorable)	45	3	23.2
Stratum II (favorable)	221	140	31.6
P. dorsalis found	41	71	35.5
P. cinereus found	45	72	31.1
No salamanders found	189	—	28.9

P. dorsalis, P. cinereus, and those without salamanders showed a significant difference ($F = 6.00$, $df = 2,220$). Plots with salamanders had a higher per cent moisture than those without salamanders. Often *P. dorsalis* was found in an area of seepage below a limestone bluff where the soil was nearly saturated. *Plethodon cinereus* was seldom found in this type of situation, but inhabited drier environments.

Food

The food of *P. cinereus* has been described by several writers (Smallwood, 1928; Hamilton, 1932; Jameson, 1944; and others). Little food study work has been published on *P. dorsalis*. Holman (1955) lists *P. dorsalis* foods as insects, spiders, mites, snails and slugs, concluding as did previous workers on *P. cinereus*, that it is an indiscriminate feeder with availability determining food items. Thurow (1955) concluded that any difference between food items of *P. cinereus* and *P. dorsalis* probably reflects habitat differences rather than food preference or feeding behavior, moisture being perhaps the most critical factor differentiating them.

The stomachs of 72 individuals of *P. cinereus* and 71 of *P. dorsalis* were examined. Five of the *P. cinereus* and 11 of the *P. dorsalis* stomachs were empty. Smallwood (1928) said that *P. cinereus* males taken during breeding season had very little food in their stomachs, but this was not the case in this study; 12 of 14 mature *P. cinereus* males taken had filled stomachs. Ten of the 11 *P. dorsalis* without food were small and immature ($N = 10$, Range = 14.7-25.6 mm., $\bar{x} = 18.45$ mm.) while two of the five *P. cinereus* (19.5 mm. and 18.6 mm.) were small and immature. The reason for the number of small *P. dorsalis* in this category may be that hatchlings of these species, but particularly *P. dorsalis*, apparently feed little if at all before hibernation (Thurow, 1955).

A summary of the stomach contents for the 67 *P. cinereus* and 60 *P. dorsalis* with filled stomachs is presented (Table 3). *Plethodon cinereus* consumed a greater variety of food items than did *P. dorsalis*. Insect material comprised approximately half of the food items in each species, although *P. cinereus* ate a significantly greater number of insects than *P. dorsalis*, 576 items to 308 items respectively (Table 1, Chi-Square = 25.90, 1 df).

Plethodon cinereus consumed more small items than did *P. dorsalis* (mites, 194 to 166 respectively; and Collembola, 324 to 162 respectively). *Plethodon cinereus* also ate a greater quantity (125 items) and variety (10 families) of Coleoptera than did *P. dorsalis* (54 items, 6 families).

Both species ate many larvae with *P. cinereus* eating more Coleoptera and Diptera larvae and *P. dorsalis* more Lepidoptera larvae.

P. cinereus ate a greater variety (13 groups to 10 groups) of non-insect materials but *P. dorsalis* ate a greater quantity (57.9% as compared to 46.3% volume), due mainly to the greater number of earthworms.

The stomachs of *P. cinereus* showed a high occurrence of seeds, but it is likely that these were accidentally taken with other food items. Although *P. dorsalis* contained fewer seeds than *P. cinereus*, it had a greater amount of vegetable material (1.97% to 0.36%) volume.

The most important food items by per cent volume (Table 4) show some differences between the species. *Plethodon dorsalis* ate significantly more earthworms (Table 4, Chi-Square = 10.02, 1 df), its major food item, than did *P. cinereus*. Coleoptera, the major food item of *P. cinereus*, was eaten in significantly greater numbers by *P. cinereus* than by *P. dorsalis* (Table 4, Chi-Square = 6.63, 1 df). This difference of beetles vs earthworms was the most striking of the food differences between the two species. Among beetles, the family Anthicidae was most important with a significant difference (Table 3, Chi-Square = 11.16, 1 df). *Plethodon cinereus* ate significantly more Collembola (Table 4, Chi-Square = 18.80, 1 df). Araneids, sixth in importance by volume to *P. cinereus*, were seldom eaten by *P. dorsalis*.

Size is surely an important factor when considering the food habits of salamanders, since as the salamander grows older it can ingest larger pray. Smaller *P. dorsalis* and *P. cinereus* ate more small food items such as mites and Collembola than the larger *P. dorsalis* and *P. cinereus* (Table 5). Chi-Square test on the size classes of both species indicate a significant difference in the consumption of mites (*P. dorsalis*, Chi-Square = 53.06, 2 df; *P. cinereus*, Chi-Square = 6.02, 2 df) and Collembola (*P. dorsalis*, Chi-Square = 40.53, 2 df; *P. cinereus*, Chi-Square = 27.30, 2 df).

Larger *P. dorsalis* and *P. cinereus* ate greater numbers of larger food items such as earthworms and Coleoptera, though Coleoptera formed a larger per cent volume in the smaller size class of *P. dorsalis* than the larger size class (Table 5). Chi-Square tests on the size classes in both species indicate a significant difference in the consumption of earthworms (*P. dorsalis*, Chi-Square = 49.97, 2 df; *P. cinereus*, Chi-Square – 23.32, 2 df) and Coleoptera (*P. dorsalis*, Chi-Square = 11.31, 2 df; *P. cinereus*, Chi-Square = 43.81, 2 df).

The small *P. dorsalis* ate more mites and Collembola than the small *P. cinereus* and, although these food items became less important in the larger size classes, the large *P. cinereus* ate more mites and Collembola than the large *P. dorsalis* (Table 5). Chi-Square tests on the smallest size class indicate no significant difference between species (mites, Chi-Square = 2.56, 1 df; Collembola, Chi-Square = 0.35, 1 df). In the largest size class *P. dorsalis* ate significantly more mites than *P. dorsalis* (Chi-Square = 4.97, 1 df), but there was no significant difference in the consumption of Collembola (Chi-Square = 0.35, 1 df).

TABLE 3. A stomach analysis of 67 *Plethodon cinereus* and 60 *P. dorsalis* from south central Parke County, Indiana, taken during the fall of 1967

	P. dorsalis			*P. cinereus*		
	Total Items	% Freq.	% Vol.	Total Items	% Freq.	% Vol.
Total insects	(308)*	(42.1)	(576)		(53.7)	
COLEOPTERA	(54)		(8.1)	(125)		(20.7)
Anthicidae	16	15.0	1.8	63	41.8	9.3
Cucujidae	1	1.7	.1	11	14.9	2.0
Histeridae	11	11.7	1.5	9	10.5	1.3
Curculionidae	1	1.7	.2	1	1.5	.2
Staphylinidae	1	1.7	.2	1	1.5	.2
Phalacridae	—	—	—	3	4.5	1.0
Chrysomelidae	—	—	—	1	1.5	.1
Elateridae	—	—	—	2	3.0	.4
Pselaphidae	—	—	—	2	1.5	.3
Dascillidae	2	1.7	.1	—	—	—
Unid. Coleoptera	16	23.3	4.3	10	11.9	1.5
Coleoptera Larvae	6	8.3	.3	22	28.4	4.5
HOMOPTERA	(7)		(1.0)	(9)		(3.2)
Cercopidae	2	3.3	.5	3	4.5	1.9
Cicadellidae	1	1.7	.3	3	4.5	.6
Aphididae	2	3.3	.1	1	1.5	.1
LEPIDOPTERA Larvae	9	13.3	4.2	10	9.0	2.8
NEUROPTERA Larvae	3	5.0	.6	1	1.5	.2
MECOPTERA	—	—	—	1	1.5	.1
Unid. Misc. Larvae	6	10.0	1.1	.11	9.0	.6
TOTAL NON-INSECTS	(293)		(57.9)	(311)		(46.3)
Earthworms	105	60.0	36.7	57	35.9	19.9
Araneida	5	5.0	.6	18	23.9	3.7
Gastropoda	4	6.7	.6	18	14.9	1.5
Isopoda	—	—	—	4	4.5	1.0
Chilopoda	1	1.7	.2	1	1.5	.1
Diplopoda	—	—	—	2	3.0	.3
Pseudoscorpionida	1	1.7	.2	4	5.9	.4
Nematoda	—	—	—	4	3.0	.1
Shed Skin	2	3.3	.4	2	3.0	.9
Misc. Animal Material	—	38.3	12.8	—	49.21	14.0
Mites	166	58.3	4.6	194	64.2	3.1
Seeds	11	10.0	.4	9	9.0	1.1
Misc. Plant Material	—	11.7	2.0	—	4.5	.4
TOTAL	(601)			(887)		
Unid. Homoptera	2	3.3	.1	2	3.0	.6
DIPTERA	(37)		(12.98)	(59)		14.1
Misc. Diptera	9	11.7	3.8	12	10.5	.5
Bibionidae Larvae	4	7.7	2.3	19	17.9	5.9
Misc. Diptera Larvae	24	40.0	6.9	28	41.8	7.7
HEMIPTERA	(10)		(2.9)	(8)		(1.8)
Tingidae	7	10.0	1.6	5	7.5	.5
Pentatomidae	2	1.7	1.0	—	—	—
Cydnidae	1	1.7	.3	—	—	—
Hydrometridae	—	—	—	1	1.5	.5
Anthocoridae	—	—	—	1	1.5	.6
Misc. Hemiptera	—	—	—	1	1.5	1.2
HYMENOPTERA	(20)		(2.4)	(28)		(2.4)
Formicidae	15	20.9	2.0	19	18.3	1.2
Misc. Hymenoptera	5	6.7	.3	9	9.0	.5
COLLEMBOLA	(162)		(8.4)	(324)		(6.2)
Smynthuridae	43	36.7	3.0	93	44.8	1.8
Entomobyridae	67	53.3	4.0	100	47.8	3.4
Poduridae	52	25.0	1.5	131	19.4	1.6

*Numbers in parentheses indicate totals for that category

TABLE 4. Major food items by per cent volume from a stomach analysis of 60 *P. dorsalis* and 67 *P. cinereus*

P. dorsalis				*P. cinereus*			
FOOD ITEM	% VOL.	% FREQ.	NO. of ITEMS	FOOD ITEM	% VOL.	% FREQ.	NO. of ITEMS
Earthworms	36.7	60.0	105	Coleoptera	20.7	66.3	125
Diptera	13.0	40.0	37	Earthworms	19.9	35.8	57
Misc. animal Material	12.8	38.3	—	Diptera	14.1	46.5	59
Collembola	8.4	58.7	162	Misc. Animal Material	14.0	49.2	—
Coleoptera	8.1	31.3	54	Collembola	6.8	72.3	324
Mites	4.6	58.3	166	Araneida	3.7	23.9	18
Lepidoptera	4.2	13.3	9	Homoptera	3.2	12.0	9
Hemiptera	2.9	11.7	10	Mites	3.1	64.2	194
Hymenoptera	2.4	27.6	20	Lepidoptera	2.8	9.0	10
Misc. Veg. Material	2.0	11.7	—	Hymenoptera	2.4	24.3	28
Unid. Larvae	1.1	10.0	6	Hemiptera	1.8	12.0	8
Homoptera	1.0	10.0	7	Gastropoda	1.5	14.9	18
Araneida	0.6	5.0	5	Seeds	1.1	9.0	9
Gastropoda	0.6	6.7	4	Isopoda	1.0	4.5	4
Shed Skin	0.4	3.3	2	Shed Skin	0.9	3.0	2
Seeds	0.4	10.0	11	Chaetopoda	0.4	5.9	4

Between the smallest size classes of the species there was no difference in the number of Coleoptera eaten. Comparing the middle and large size classes, *P. cinereus* ate significantly more Coleoptera than *P. dorsalis* (Chi-Square = 5.27, 1 df) and *P. dorsalis* ate significantly more earthworms (Chi-Square = 15.54, 1 df).

TABLE 5. A comparison of food items eaten in different size classes of *P. dorsalis* and *P. cinereus*

	P. dorsalis			*P. cinereus*		
	10-24 mm. (24)*	25-34 mm. (14)	35+ mm. (22)	10-24 mm. (21)	25-34 mm. (24)	35+ mm. (22)
SMALL ITEMS						
Collembola						
% Vol.	18.4	3.1	.9	11.5	7.3	3.9
% Freq.	75.0	57.1	27.3	57.1	54.2	42.7
No. Items	89	82	32	103	32	27
Mites						
% Vol.	9.6	2.6	.4	6.0	2.4	1.1
% Freq.	75.0	71.4	31.2	76.2	66.7	50.0
No. Items	108	37	21	68	79	47
LARGE ITEMS						
Coleoptera						
% Vol.	9.2	6.9	6.5	9.0	23.3	21.6
% Freq.	7.9	18.2	14.1	19.6	64.2	50.8
No. Items	8	13	37	7	35	63
Earthworms						
% Vol.	17.2	35.7	58.7	14.3	18.3	27.0
% Freq.	37.5	64.3	81.2	28.6	29.2	50.0
No. Items	9	28	68	8	13	36

*Figures in parentheses represent number of individuals in each size class

A comparison between per cent items in stomachs and per cent items in Berlese samples was used to determine relative predilection, but it has shortcomings since many factors important to salamanders, such as size of an organism, its ability to escape, or its occurrence in a humus layer different from the one that the salamander feeds in, are not reflected in funnel extractions. For example, the habitat samples contained large percentages of Chilopoda and Araneida while only small percentages were found in the stomach samples because the large size of many of them may have discouraged predation. Further, the efficiency of the sample method is undetermined (Murphy, 1962). Accuracy may be lessened because it fails to extract inert stages, predation may occur during the drying period, and unknown factors may reduce the extraction of other organisms. The discrepancy between the small percentage of earthworms and the large percentage found in stomachs indicate an inefficient extraction technique for them.

To determine selectivity, a comparison index was calculated using per cent of stomach items and per cent of Berlese sample items. The larger value was divided by the smaller for each item. An index value of one indicates that items in the stomachs and in the habitats were the same. Positive values indicate that items occurred more often in stomachs and negative values that items occurred more often in Berlese samples (habitats).

Because of the inefficiencies of the Berlese method in reflecting the items available to the salamander, the comparison index does not accurately reflect the sensitivity of a species and must be weighed lightly. It can be used to show differences in selection between species since Berlese samples for both species are comparable, by examining the comparison index for both species. A higher value indicates that the species takes a larger per cent of the items available, thus showing a stronger selection for the item than the other species (Table 6).

TABLE 6. A comparison in per cent of items in stomachs to items in the habitat (Berlese funnel samples) for *P. dorsalis* and *P. cinereus* Comparison index = higher per cent items divided by lower per cent item. Positive values indicate higher per cent items in the stomach, negative values indicate higher per cent items in the Berlese sample.

	P. dorsalis			*P. cinereus*		
Food Item	% of Stomach Items	% of Berlese Items	Comparison Index	% of Stomach Items	% of Berlese Items	Comparison Index
Mites	26.56	51.77	−1.94	21.17	58.13	−2.
Collembola	25.92	3.70	7.00	35.36	4.05	8.
Earthworms	16.80	——	——	6.22	0.27	23.
Diptera	12.16	3.36	3.62	10.69	6.90	1.
Coleoptera	8.16	−2.49	13.68	13.68	11.83	1.
Hymenoptera	3.20	3.03	1.06	3.05	1.62	1.
Hemiptera	1.60	1.50	1.07	.84	2.30	−2.
Lepidoptera	1.44	0.32	4.50	1.09	——	——
Homoptera	1.28	0.84	1.52	0.95	0.67	1.
Unid. larvae	0.96	——	——	1.20	——	——
Spiders	0.80	13.15	−16.44	1.96	9.62	−4.9
Gastropoda	0.64	0.32	2.00	1.96	——	——
Centipede	0.16	1.85	−11.56	.10	3.11	−31.10
Pseudoscorpion	0.16	——	——	0.43	0.40	1.08
Neuroptera larvae	0.16	——	——	0.32	——	——
Isopoda	——	——	——	0.42	——	——
Diplopoda	——	——	——	0.21	——	——
Mecoptera	——	——	——	0.10	——	——

Mites are more numerous in *P. cinereus* habitats than *P. dorsalis* habitats (58.13% and 51.77% respectively) although eaten more often by *P. dorsalis* (26.56%) than *P. cinereus* (21.17%). Thus *P. dorsalis* shows a greater selectivity for mites than does *P. cinereus*.

Coleoptera, although found more frequently in *P. dorsalis* habitat that in *P. cinereus* habitat (20.30% and 11.83% respectively), were more often eaten by *P. cinereus* than *P. dorsalis* (13.68% and 8.16% respectively), therefore indicating that *P. cinereus* selected for Coleoptera more than *P. dorsalis* did. Further *P. dorsalis* selects more Diptera, Hemiptera, and mites than does *P. cinereus, but P. cinereus* selects more Coleoptera, Araneida, Hymenoptera and Collembola. Items eaten only by one species or not appearing in Berlese samples were not comparable in this manner.

Further indications of differences in selection of items by the two species could be found by comparing the stomach contents of salamanders that occurred together, since both would be selecting from the same organisms. Examination of Berlese data from those plots in which both species occurred showed significant differences in the occurrence of Coleoptera (Chi-Square = 5.12, 1 df) and mites (Chi-Square = 6.36, 1 df).

Differences in the fauna surrounding the top species may indicate habitat differences better than measurement of physical characteristics since the combination of factors present affects each salamander and its surrounding fauna similarly. Comparison of Berlese funnel collections from capture sites of the two species suggests through the different fauna associated with each species that habitat differences do exist (Table 6). Diptera and Hemiptera were about twice as numerous in the habitat of *P. cinereus*, while Coleoptera and Hymenoptera were over twice as numerous in the *P. dorsalis* habitat.

TABLE 7. A comparison of per cent food items and per cent Berlese sample items for *P. dorsalis* and *P. cinereus* found in the same plots and in separate plots

Species	Coleoptera		Mites		Collembola	
	% Items	No. Items	% Items	No. Items	% Items	No. Items
P. cinereus w/*P. dorsalis* (26)*						
Stomach items	17.0	64	23.9	90	32.2	121
Berlese items	12.9	69	59.9	561	3.8	21
P. dorsalis w/*P cinereus* (28)						
Stomach items	8.8	18	25.6	70	22.3	69
Berlese items	23.9	120	52.7	349	3.6	19
P. cinereus (41)						
Stomach items	12.2	69	22.0	125	35.7	203
Berlese items	10.3	97	57.2	308	4.3	40
P. dorsalis (34)						
Stomach items	7.6	24	25.2	80	24.9	79
Berlese items	17.5	116	47.2	247	3.8	25

*Numbers in parentheses indicate the number of specimens in each group.

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Nesting Silver Lampreys, *Ichthyomyzon unicuspis*, in the Little Blue River (Southern Indiana, Crawford County, Ohio River Drainage)

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ABSTRACT

On May 13, 1987, we located a nesting and spawning aggregation of silver lampreys, *Ichthyomyzon unicuspis*, on a gravel-bottomed riffle in the Little Blue River, a southern Indiana tributary of the Ohio River. The only other definite spawning locality for this species in the Ohio River drainage is Sunfish Creek in Pike County, Ohio. Few modern records are available for silver lampreys in the Indiana section of the Ohio River. In adjacent states, population declines have been reported in streams with habitat destruction and impoundments. In Indiana waters, additional research is needed to locate other nesting sites so that management procedures may be developed to protect existing spawning sites.

Introduction

The purpose of this paper is to report the first nesting and spawning aggregation of silver lampreys, *Ichthyomyzon unicuspis*, observed in Indiana waters tributary to the Ohio River. The only other definite spawning locality for this parasitic species in the entire Ohio River drainage is Sunfish Creek in Pike County, Ohio.[6]

Site Description

On May 13, 1987, some nine nesting adults in various color phases from a prespawning yellow-tan to a spawning blue-silver were found in the Little Blue River clinging to rocks in the first gravel-bottomed riffle complex south of the U.S. Highway 62 bridge in Crawford County about three miles east of Sulphur, Indiana. One spawning female in the blue-silver color phase was preserved as a voucher specimen.

Background Information

The widely distributed silver lamprey has a reported range from the Hudson and St. Lawrence drainages westward through the Great Lakes, southward into the Upper Mississippi and eastward into the Ohio River drainage.[3] Historical information indicates that silver lampreys were extremely abundant, especially in Lake Erie, prior to 1875.[6] Thereafter, significant population declines have been reported in adjacent Ohio and Indiana where habitat alterations have destroyed former nesting sites, impoundments have prevented upstream migrations, and increased turbidity and siltation have hindered the hatching of unprotected eggs and later impaired feeding and development of the ammocoetes larvae.[5,6] In the Ohio drainage, knowledgable individuals like commercial fishermen have noted significant decreases in abundances of all parasitic lampreys.[6] In Indiana waters, so few modern records are available[2] that Nelson and Gerking in 1968 listed the presence of silver lampreys in the Ohio drainage as doubtful.[4] In northern Indiana, the species evidently no longer occurs in Lake Michigan and its tributaries.[5]

Discussion

Water managers and individuals reviewing proposals for stream alteration projects should be cognizant of the silver lamprey's fragile life history. Such an increased awareness may help mitigate possible losses in the future. For example, when stream alterations are being considered, sampling of backwater bars and areas of suitable larval habitat should be undertaken to determine the presence or absence of ammocoetes larvae. The reason for collections in slack water is that following hatching and downstream drifting to backwater areas, the larvae develop U-shaped tubes where they feed upon microscopic organic debris for a period of four to seven years.[1,3] Next, collections and observations of spawning adults should be timely and correlated with the short nesting phase of the life cycle. After a parasitic life of only 10 to 20 months[3], sexually mature silver lampreys migrate upstream in early spring to riffle nesting sites where they spawn and die shortly thereafter. We recommend that additional research be undertaken during the month of May to locate other nesting sites, if any, in Indiana waters so that appropriate management procedures can be developed to protect existing spawning areas.

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Influence of Tillage Practices on the Diets of Deer Mice

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Introduction

Small mammal populations in cultivated ecosystems have received limited research attention (11, 15, 20, 23, 28). Because of the tremendous amount of acreage devoted to agricultural crops, especially in the midwestern United States, these ecosystems and the animals that utilize them deserve study. Additionally, major shifts in farming practices may dramatically impact wildlife that utilize these early successional habitats. One such change is the recent and ever increasing adoption of zero tillage or no-till and a variety of other tillage practices, collectively referred to as conservation tillage. All have in common reduced disturbance of the soil surface resulting in the retention of greater amounts of surface residues. The impacts of conservation tillage practices on small mammals have only recently been addressed and have been concerned primarily with species occurrence, population levels, and damage to crop seedlings by rodents (7, 8, 13, 19, 25, 30).

The deer mouse (*Peromyscus maniculatus*) is generally the most abundant small mammal, and many times the only permanent resident species, inhabiting row-crop fields over much of the United States (7, 8, 11, 13, 20, 25). Although food habits of deer mice have been described for a variety of habitats and locations (5, 9, 10, 12, 17, 18, 21, 24, 29), few studies have been conducted in corn and soybean fields where tillage methods are detailed (8, 13, 16, 27). The purposes of our study were to document summer and winter foods of deer mice in agricultural fields and to explore the influence of crop and tillage practices on diets.

Study Area and Methods

During 1983 and 1984, deer mice were captured from 36 winter (January-February) and 35 summer (June-July) row-crop fields in Scott Co., southeastern Indiana. All fields were on privately owned farms. The topography is flat to moderately rolling with silt loam soils derived from glacial till. Cultivated fields were generally irregular in shape due to topography and numerous small waterways. Fields surveyed in winter and summer averaged 14.4 ha and 10.3 ha, respectively.

Winter fields were classified into four categories: corn residue, soybean residue, tilled, or fallow (Table 1). Corn residue fields had been planted to corn the previous summer and residues left unchopped after fall harvest. Of these, five fields also had an aerially seeded cover crop (wheat, ryegrass, hairy vetch, or crown vetch) present. Seven of the soybean residue fields had been conventionally planted to soybeans the previous summer, three had wheat residue due to double cropping, one had been no-tilled into corn residue, and one had a cover crop of crown vetch. Tilled fields included six disced corn fields, one chisel-plowed corn field, one moldboard-plowed corn field, and two chisel plowed soybean fields. The fallow fields were sampled only in 1984 and had been entered in the U.S. Department of Agriculture Payment-

TABLE 1. Dear mice examined for food habits from Indiana row-crop fields, 1983 and 1984.

Fields		Mice captured			
Type	N	Trap-nights	N	$\bar{X}$	Range
Winter					
Corn residue	11	3200	70	6.4	1-12
Soybean residue	12	3000	66	5.5	1-10
Tilled	10	3400	117	11.7	2-31
Fallow	3	600	39	13.0	4-24
Summer					
Tilled corn	7	2200	36	5.1	2-11
Tilled soybeans	9	2600	89	9.9	4-22
No-till corn	7	1400	50	7.1	1-18
No-till soybeans	8	1600	28	3.5	1-7
Fallow	4	800	35	8.8	3-22

In-Kind (PIK) program the previous summer (6). Each PIK field had been previously planted to corn, contained cover crops (wheat, sweet-clover, ryegrass, or crown vetch), and had been mowed in the fall.

Summer fields were classified into five categories: tilled corn, tilled soybeans, no-till corn, no-till soybeans, or fallow fields (Table 1). Tilled fields had been conventionally prepared for planting by plowing or discing in the spring resulting in less than 15% surface residues. No-till fields were slot-planted into existing residues following applications of fertilizers, herbicides, and, sometimes, insecticides. Conventionally tilled corn fields had been planted to corn (five fields) or soybeans (four fields) or had been fallowed (one field). No-till corn fields were established in fields that contained corn residue (three fields), soybean residue (one field), soybean-wheat residue (one field), or had been fallowed (two fields). Five of the no-till soybean fields followed winter wheat harvest. The remaining were slot-planted into corn residue (two fields) or soybean residue (one field). The fallow fields were studied during their inclusion in the PIK program in 1983. All had been planted previously to corn, and three had cover crops.

Deer mice were captured in snap traps placed at 10-m intervals along linear transects extending from the field edge to the field interior. Transects contained 25 traps, and two or four transects were established in each field. Traps were attended for four consecutive nights so trapping effort totaled 18,800 ($\bar{X}$ = 265) trap-nights (Table 1). Summer trapping began 13-74 days ($\bar{X}$ = 37) after crops were planted. Traps were baited with peanut butter or a peanut butter-rolled oats mixture. Captured mice were immediately frozen, later thawed, and the stomachs removed and placed in formalin or ethanol until contents were examined. Stomach contents from each mouse were segregated into similar items, and the percent volume represented by each item was estimated visually. The mean percent volume for each food item was calculated; i.e., the aggregate percentage method (22) was used. Stomachs less than half full were discarded. Items were identified to species when possible but in many cases classification was to animal order or class, or plant family. The total number of different foods present in each stomach was used as a measure of dietary diversity and statistically analyzed using Chi-square tests and analysis of variance of a nested factorial design. Relative frequency of occurrence of the major food categories (cultivated grains, other seeds, invertebrates, and miscellaneous) also was calculated and used to test statistical differences in diets using Chi-square tests.

Relative availability of potential deer mouse foods was not determined because sampling radically different foods to make accurate comparisons among food types

is difficult. Furthermore, it also is difficult to decide what items are acceptable foods and under what circumstances they are available to deer mice.

Results

Deer mice utilized a large variety of foods (Table 2) attesting to their highly omnivorous habits. Seeds other than cultivated grain included plants of the genera *Cerastium, Oxalis, Setaria, Capsella*, and *Prunus*. Invertebrate foods were mostly insects that included the following families: Scarabaeidae, Gryllidae, Chrysomelidae, Acrididae, Tupulidae, Lygaeidae, Carabidae, Formicidae, Curculionidae, and Otitidae. *Solanum* fruit and *Endogone* (fungi) were minor miscellaneous items.

TABLE 2. Percent volume of food items identified from deer mouse stomachs in Indiana row-crop fields, 1983 and 1984.

Item	Winter (N = 292)		Summer (N = 238)	
Grain	71.9		32.5	
Corn		38.3		5.1
Soybeans		25.3		18.3
Wheat		3.9		9.1
Unidentified		4.3		0.0
Seeds	4.8		26.9	
Grass		2.9		7.8
Forbs		0.7		15.4
Unidentified		1.3		3.7
Invertebrates	13.9		39.4	
Insecta (larva)		7.4		18.1
Coleoptera		2.4		9.0
Lepidoptera		2.4		8.5
Diptera		1.8		0.7
Unidentified		0.8		0.0
Insecta (adults)		2.6		15.4
Orthoptera		0.9		7.0
Coleoptera		0.6		3.7
Lepidoptera		0.0		1.1
Hemiptera		0.2		0.7
Diptera		<0.1		0.5
Trichoptera		0.0		0.3
Hymenoptera		<0.1		0.2
Homoptera		<0.1		0.0
Unidentified		0.7		2.0
Oligochaeta		1.5		2.1
Gastropoda		1.6		0.0
Arachnida		0.6		3.0
Chilopoda		0.1		0.1
Nematoda		0.0		0.1
Unidentified		<0.1		0.5
Miscellaneous	9.4		1.2	
Green vegetation		6.4		0.7
Fleshy fruit		2.3		0.1
Fungi		0.0		<0.1
Vertebrate flesh		0.8		0.5

Diets of deer mice, as reflected by stomach contents, showed marked seasonal differences (Figure 1, 2). Waste grain was an important food during both periods, but it was found much more frequently during the winter ($X^2 = 92.5$, df = 1, P

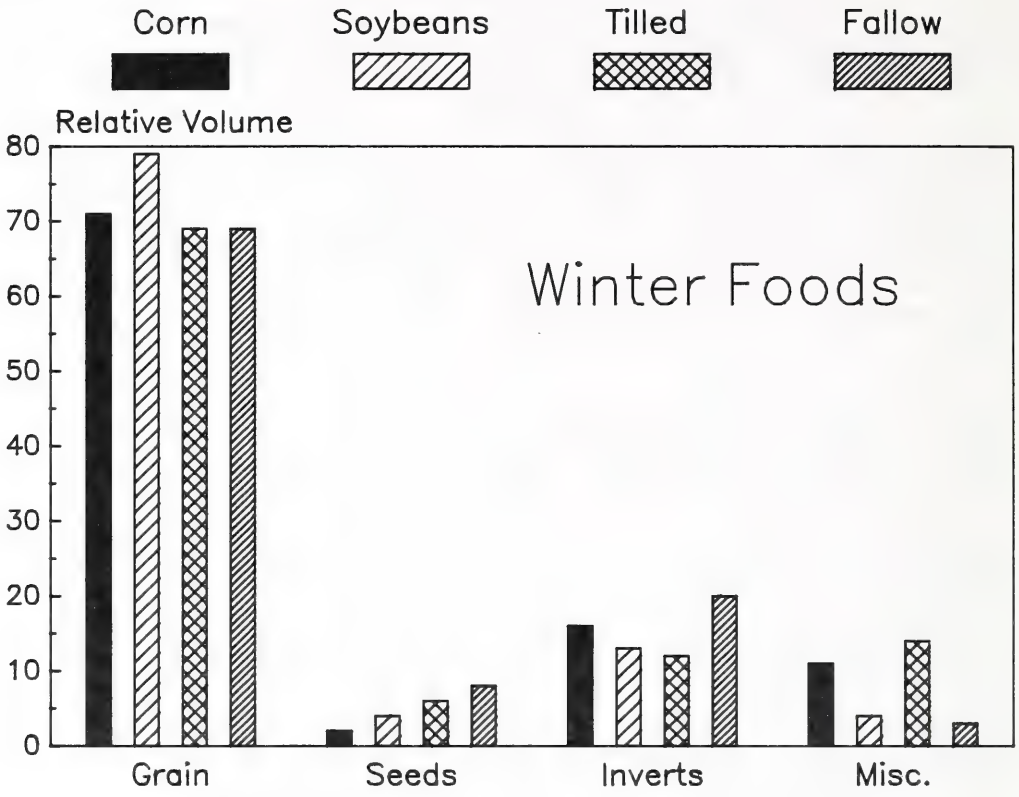

FIGURE 1. Percent volume of foods from deer mice during winter in southeastern Indiana row-crop fields, 1983 and 1984.

< 0.001). During summer, invertebrates ($X^2 = 76.6$, df = 1, $P < 0.001$) and seeds ($X^2 = 83.4$, df = 1, $P < 0.001$) were much more important than during winter. Seeds were a relatively minor component of winter diets. Miscellaneous items were consumed more frequently during the winter ($X^2 = 18.4$, df = 1, $P < 0.001$).

In contrast to distinct seasonal differences, occurrences of major food items among crop and tillage types were similar within each season. In winter, the frequency of grain ($X^2 = 3.5$, df = 3, $P > 0.05$) and invertebrates ($X^2 = 4.1$, df = 3, $P > 0.05$) did not differ appreciably among field types (Figure 1). Statistical differences among tillage categories, however, did exist for seeds ($X^2 = 9.6$, df = 3, $P < 0.05$) and miscellaneous foods ($X^2 = 15.8$, df = 3, $P < 0.001$). These differences were relatively minor, however, and were due primarily to a greater frequency of occurrence of seeds in stomachs from idled fields and a greater than expected occurrence of miscellaneous items from mice in corn residue and tilled fields.

Dietary variation attributable to field types was greater for the summer period (Figure 2). Differences in the frequency of grain eaten by deer mice ($X^2 = 16.5$, df = 4, $P < 0.01$) are due mostly to no-till soybean fields where wheat was eaten much more than other foods, probably reflecting the super abundance of wheat resulting from double cropping. Statistical differences in the occurrence of seeds ($X^2 = 44.4$, df = 4, < 0.001) are due to their greater frequency in mice from fallowed fields coupled with lower frequency in mice from tilled soybean and tilled corn fields. Invertebrates differed ($X^2 = 25.0$, df = 4, $P < 0.001$) by being found more often in mouse stomachs from tilled soybean fields and less often from no-till soybean

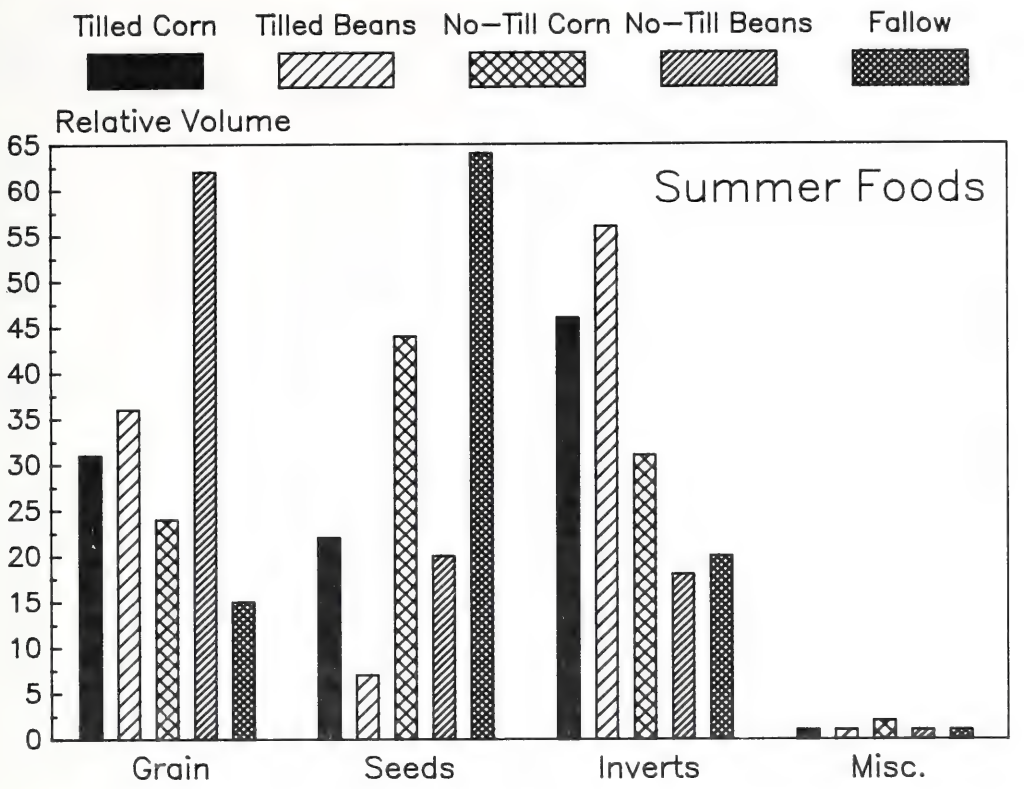

FIGURE 2. Percent volume of foods from deer mice during summer in southeastern Indiana row-crop fields, 1983 and 1984.

fields. Miscellaneous items showed no discernible differences ($X^2 = 2.6$, df = 4, $P < 0.05$) among field types.

The mean (±SE) number of different food items tallied in deer mouse stomachs showed consistent seasonal differences, but minor differences among field types within a season. Deer mouse diets during winter contained 1.52 ± 0.05 items compared to 2.13 ± 0.07 items during summer ($X^2 = 60.0$, df = 3, $P < 0.001$). During the winter period, means ranged from 1.44 ± 0.16 items/stomach for tilled fields to 1.77 items for fallowed fields. Corn (1.50 ± 0.08) and soybean (1.53 ± 0.09) residue fields had intermediate values, but no statistical differences could be shown using analysis of variance ($F = 2.04$; df = 3, 28; $P > 0.05$) or Chi-square analysis ($X^2 = 8.68$, df = 6, $P > 0.05$).

The mean (±SE) number of items/stomach for summer samples was greatest for tilled soybean (2.21 ± 0.11) and corn (2.19 ± 0.14) and lowest for no-till corn (2.02 ± 0.14) and soybean (2.04 ± 0.22) fields. Fallowed fields had an intermediate value (2.11 ± 0.19), but no statistically significant difference could be detected among field types ($F = 0.35$; df = 4, 26; $P > 0.05$) ($X^2 = 7.64$, df = 23, $P > 0.05$).

Discussion

Dear mice inhabiting corn and soybean fields take advantage of the readily available waste grain present, especially during winter. The absolute abundance of some food items has been shown to be affected by tillage practices. Fall discing and deep plowing reduced waste corn in Texas by 77% and 97%, respectively (1). Warner

et al. (26) found similar levels of waste grain reductions from fall tillage in Illinois corn and soybean fields. Weed seeds may follow a similar pattern of reduction, but seed abundance at harvest is influenced primarily by weed control using herbicides and mechanical cultivation during the growing season (3).

Patterns of arthropod abundance due to tillage practices are less clear than for waste grain. Basore (2) found comparable numbers of insects in Iowa no-till and conventionally tilled fields during summer. Other researchers have reported greater abundance and diversity of invertebrates in no-till fields (4, 14, 25). Tillage and the use of insecticides may have only temporary impacts on arthropod densities. The basic similarity of diets of deer mice within each season during our study suggests either that different food items are not seriously limited, each field type has roughly similar relative amounts of food available, or that deer mice actively select food items in order to balance their diets. Without accurate information on food availability, these hypotheses cannot be tested.

Comparisons of deer mouse food habits among different studies are tenuous because of differences in methodology, timing, and site characteristics. Post-planting samples of deer mouse stomachs from studies in Iowa (8) and Nebraska (13) no-till cornfields, showed a greater preponderance of inverteberates and herbaceous items than our study. Seeds other than grain were considerably more prevalent in the diets of deer mice inhabiting Indiana cornfields. The frequency of occurrence of corn was similar in the Indiana and Iowa studies, although other cultivated grains were detected more frequently in mice from Indiana. Corn was a more important constituent in Nebraska deer mice, but the amount was comparable to total grain from mice in Indiana no-till cornfields.

Summary

Stomach contents from 530 deer mice from southeastern Indiana corn and soybean fields were examined in 1983 and 1984. During winter, 292 mice were collected from 36 fields categorized as corn residue, soybean residue, tilled, or fallow. Major food items summarized as relative volume using the aggregate percentage method, were waste grain (72%), invertebrates (14%), seeds (5%), and miscellaneous (9%). Diets were similar among field types although seeds occurred more often in fallow fields, and miscellaneous foods were more prevalent in corn residue and tilled fields. During summer, 238 stomachs were examined from 35 fields classified as tilled corn, tilled soybeans, no-till corn, no-till soybeans, or fallow. Diets differed noticeably from the winter season and consisted of invertebrates (39%), grain (33%), seeds (27%), and miscellaneous (1%). Field types influenced diets to a greater extent in summer than in winter. The frequency of grain eaten by deer mice was greatest in no-till soybean fields, which were primarily double-cropped fields containing abundant wheat left after harvest. Intevertebrates appeared in diets disproportionately more often in tilled soybean fields and less often in no-till soybeans. Seeds were again more prevalent in mice from fallow fields and encountered less frequently from those in tilled soybean fields.

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A History of the Zoology Section of the Indiana Academy of Science

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The first volume of the *Proceedings of the Indiana Academy of Science*, published in 1892, included a listing of 460 papers that were read before the Academy between 1885 and 1891. At least 172 of these papers, or 37%, were zoological in nature. A total of 26 titles was listed for O.P. Hay, 19 for David Starr Jordan, 18 for Amos W. Butler, 14 for Carl W. Eigenmann, 12 for O.P. Jenkins, and 11 for Barton W. Evermann. The most popular topic was fish which was the subject of at least 55 papers.

For the first 15 years of the Academy history (1885-1900), 88 zoologists presented more than 386 papers. The list was headed by Carl H. Eigenmann, who gave at least 50 papers, followed by A.W. Butler with 40, O.P. Hay with 26, and B.W. Evermann with 25.

At first all papers were read before the Academy in a general session. Beginning in 1888, papers could be read in a general session or in one of four sections which included Zoology, Botany, Chemistry-Physics-Mathematics, and Geology-Geography. By 1892, the sections were abolished and all papers were presented in general sessions, with the papers arranged according to subject matter. Generally the two categories were in biological sciences and the physical sciences.

The degree of separation into separate subject headings depended on the total number of papers submitted. For example, in 1907 there were 14 papers in a "section" on zoology, 4 in botany, 5 in geology, 2 in physics, and 4 in chemistry. Some years all papers were presented in one general section; other years there were two major sections, the Biological Sciences and the Physical Sciences.

It is not clear just when the Sections became permanently separated, but the first Chairman for the Zoology Section was noted in the program for 1927. That first chairman was Amos W. Butler. At least from this date the Zoology Section has been a regular feature of the Annual Fall Meetings.

A listing of the Chairmen of the Zoology Section since 1927 gives a good sampling of the more active zoologists in the state. In addition to Amos W. Butler, the list includes Will Scott for 1928; B.H. Grave, 1929; W.P. Morgan, 1931; N.E. Pearson, 1932; B.H. Grave, 1933; Fred Donaghy, 1934; J.P. Scott, 1935; B.E. Montgomery, 1936; W.P. Allyn, 1937; Henry G. Nester, 1938; C.P. Hickman, 1939; W.A. Hiestand, 1940; W.E. Martin, 1941; T.M. Sonneborn, 1942; R.M. Cable, 1943; W.H. Headlee, 1944; W.R. Brenneman, 1945; Howard H. Vogel, 1946; A.E. Reynolds, 1947; John W. Baechle, 1948; M.R. Garner, 1949; Sears Crowell, 1950; C.J. Goodnight, 1951; F.R. Elliott, 1952; W.H. Johnson, 1953; John D. Mizelle, 1954; Jackson Webster, 1955; Enos G. Pray, 1956; Eliot Williams, 1957; William Hopp, 1958; Forst Fuller, 1959; Carl H. Krekeler, 1960; L.E. DeLanney, 1961; James B. Cope, 1962; William R. Eberly, 1963; Robert H. Cooper, 1964; William R. Eberly, 1965; Russell Mumford, 1966; J. Hill Hamon, 1964; John O. Whitaker, Jr., 1968, James C. List, 1969; Ralph D. Kirkpatrick, 1970; William J. Brett, 1971; Dorothy Adalis, 1972; Les Hearson, 1973; Charles E. Mays, 1974; John M. Ferris, 1975; Thomas Joseph, 1976; John O. Whitaker, Jr., 1977; Jackson L. Marr, 1978; Richard C. McCracken, 1979; Robert R. Pinger, 1980; Larry Ganion, 1981; David Sever, 1982; Ronald L. Richards, 1983;

Thomas Fogle, 1984; James R. Litton, Jr., 1985; Ronald L. Richards, 1986; and Albert A. Williams, 1987.

As zoologists became more numerous and the science of zoology more diversified, new sections were created. The first mutation to arise from the Zoology Section was the Entomology Section, which first appeared as a permanent section in 1947. A.M. Vance of Purdue University was the first chairman. Professional entomologists had been meeting in Indiana annually since 1921, chiefly at Purdue University. Later the Entomologist's meetings were held in conjunction with the meetings of the Academy. In 1946, the papers read at the Entomologist's meeting were published in a special section of the *Proceedings of the Indiana Academy of Science*. The Entomology Section has been a regular feature since 1947.

Later sections formed to accommodate the specialized interests of some zoologists include the Ecology Section in 1966, Cell Biology Section in 1967, Molecular Biology in 1969, and Environmental Quality in 1979. I'm sure some zoologists were active in the Science Education Section formed in 1970.

At the centennial meeting of the Academy in 1984, a total of 24 titles was listed for the Zoology Section. In addition, 41 zoological (non-human) papers were presented in other sections, including 1 in Anthropology, 4 in Cell Biology, 12 in Ecology, 16 in Entomology, 1 in Environmental Quality, 5 in Microbiology and Molecular Biology, and 2 in Psychology.

Zoology is still alive and well in Indiana.

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Effects of Sidestream Smoke on Reproduction and Development of Two Inbred Murine Strains

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Introduction

Since publication of the classical paper by Simpson in 1957 (47), many studies have examined the effects of smoking on maternal, fetal, and neonatal health. Infants born to women who smoke have a higher prevalence of signs suggesting intrauterine growth retardation (22). On average, the offspring of smoking mothers are 200 g or more lighter at term than those of nonsmoking mothers (47, 2). Women who smoke also have a higher prevalence of bleeding during pregnancy and delivery complications such as abruptio placentae, placenta previa, premature and prolonged rupture of membranes, spontaneous abortion, and perinatal mortality (34, 25, 55). Decreased placental blood flow (26), improper development of fetal coronary arteries (29), congenital heart disease (18), childhood respiratory ailments (9, 20, 8), and behavioral abnormalities of offspring (35) are other effects associated with maternal smoking.

Various animal studies have shown many of the same effects observed in humans. These include reduced birth weight (14, 41, 6), and increased perinatal morality (14).

There is growing evidence of health risks due to sidestream smoke (passive smoking), especially in infancy and early childhood (27, 16, 56). The issue of passive smoking is perhaps most cogent in children who are constantly subjected to smoke at home (8).

Most animal smoke-exposure studies have involved smoking machines that release either mainstream smoke (6, 32) or a combination of mainstream and sidestream smoke (24) in regulated, periodic puffs, or have exposed animals to different components of cigarette smoke (5, 39, 13, 48, 52). However, a few animal studies have involved sidestream smoke exclusively, and various harmful fetal and neonatal effects have been noted (54, 33). This study uses the same procedure and animal model used in a previous investigation (33) in an attempt to reproduce the reported effects of sidestream smoke exposure, and to determine whether or not strain variation occurs regarding these effects.

Materials and Methods

Adult female mice of the BALB/cAnNHsd and C57BL/6NHsd strains (Harlan-Sprague Dawley, Indianapolis, IN) were randomly assigned to experimental and control groups and mated overnight with males of the same strain. The day a copulation plug was found was designated as gestation day 1. Pregnant mice were maintained in separate cages under environmental conditions controlled with respect to room temperature (20-25 C), humidity (55-60%), and photoperiod (12 hr light, 12 hr dark). They were fed Lab Blox pellets (Wayne Feed Division, Continental Grain Co.) and water *ad libitum* between trials.

The experimental procedure used in this study is similar to that used in a previous study (33). Experimental mice were placed in a plexiglas smoking chamber (25.5 cm $\times$ 30.5 cm $\times$ 21.0 cm) containing 6 airholes 6.5 mm in diameter on two opposite sides, and exposed each day of gestation (21 days) to the sidestream smoke from one filter-tip cigarette. The duration of each smoking session was 40 minutes. This provided a standard time period and insured that the cigarette had burned completely. Control mice were divided into two groups: sham controls and shelf controls. Sham

controls were placed in similar smoking chambers each day for 40 min, but were exposed to an unlit rather than a lit cigarette. Shelf controls remained in their respective cages throughout the gestation and weaning periods.

At the end of each treatment period, dams were returned to their respective cages. Treatment was terminated at parturition, and the dams (P generation) were allowed to nurture their litters (F1 generation) until they were weaned on postnatal day 21. On postnatal day 1 (within 16 hr of birth) all litters were sexed, weighed, and dead fetuses removed. Cages were observed daily and offspring deaths recorded. At the end of the 21-day weaning period, the pups were again sexed.

Upon reaching sexual maturity, control and experimental F1 females of both strains were mated with males of their own strain and exposed to the same treatment format as their mothers. Both parental and F1 litters of the two strains were observed for intrastrain and interstrain effects of sidestream smoke exposure.

In a parallel study, carboxyhemoglobin (COHb) concentrations of peripheral blood (vena cava) of female P and F1 generation (gen) mice, subjected to the same conditions described above, were determined using a Model 282 CO-Oximeter (Instrumentation Laboratories, Lexington, MA) and taken as an index of the amount of smoke inhaled. Since there was no significant difference in COHb values between P gen and F1 gen mice of either strain, results are reported as pooled means ± S.E. Chi-square analysis was used to evaluate percent mortality data. Differences between means were evaluated using Student's *t* test and a P value of less than 0.05 was considered to be significant. Sham mice are the controls of reference. Statistical analysis indicated no significant difference between shelf and sham control groups.

Results

A 40 min exposure to sidestream smoke from one filter-tip cigarette produced a significant increase in the levels of carboxyhemoglobin (COHb) over background levels. BALB smoke-exposed dams had a mean COHb value of 28.33% (±3.39; N=24). Control dams had a COHb concentration of 2.15% (±0.33; N=14). Experimental C57 dams had a mean value of 30.68% (±2.32; N=24) and control dams had a mean value of 3.81% (±0.25; N=15). COHb levels of experimental dams of both strains returned to control range values within a 24 hr period (Table 1).

TABLE 1. Carboxyhemoglobin extinction data for BALB/c and C57BL mice following 40 min (0.7 hr) exposure to sidestream cigarette smoke.

Strain	N	Time (hr)	COHb (%)[1]
BALB/c	14	0.0	2.2 (±0.33)
	24	0.7	28.3 (±3.39)
	5	1.7	9.0 (±0.84)
	5	2.7	3.1 (±0.12)
	4	24.7	2.4 (±0.23)
C57BL	15	0.0	3.8 (±0.25)
	24	0.7	30.7 (±2.32)
	5	1.7	9.7 (±1.05)
	4	2.7	3.6 (±0.18)
	5	24.7	3.1 (±0.25)

1. Mean ± S.E.

Sidestream smoke produced significant but varied effects on both strains (Table 2). It reduced litter size, lowered off-spring birth weight, and increased offspring mortality of BALB parental generation (P gen) mice. A decrease in litter size and an increase in offspring mortality were observed in both C57 P gen and F1 gen mice.

Table 2. Effects of sidestream smoke on mouse litter size[1], birth weight (g), and survivorship to postnatal day 21. Number of litters given in parentheses.

					Survivorship			
Strain	Gen	Group	Litter Size	Birth Weight	Surv/ Total	%	M21/M1 (%)	F21/F1 (%)
BALB/c	P	Con (28)	7.2 + 0.50	1.58 ± 0.02	180/210	89.1	85/101 (84.2)	95/101 (94.1)
		Exp (26)	5.6 ± 0.56[2]	1.50 ± 0.03[2]	111/146	76.0[3]	47/65 (72.3)	64/81 (79.0)
	F1	Con (36)	7.3 ± 0.42	1.58 ± 0.03	235/263	89.0	106/124 (84.8)	129/138 (98.5)
		Exp (29)	6.8 ± 0.52	1.55 ± 0.02	161/197	81.4[4]	71/96 (74.0)	90/101 (89.1)
C57BL	P	Con (63)	7.4 ± 0.26	1.23 ± 0.21	295/463	63.7	140/237 (59.2)	155/226 (68.6)
		Exp (27)	6.3 ± 0.40[2]	1.18 ± 0.02	82/170	48.0[3]	45/86 (52.3)	37/84 (44.1)
	F1	Con (30)	7.4 ± 0.36	1.23 ± 0.04	146/221	66.1	66/110 (60.0)	80/111 (72.1)
		Exp (29)	6.2 ± 0.32[2]	1.23 ± 0.02	61/181	33.7[3,5]	28/92 (30.4)	33/84 (39.3)

1. Within 16 hr of parturition; equivalent to postnatal day 1.
2. Significantly different from control group, $P < 0.05$.
3. Significantly different from control group, $P < 0.01$.
4. Significantly different from control group, $P < 0.02$.
5. Significantly different from P gen exp group, $P < 0.01$.

Control and experimental mice of both BALB P and F1 generations had significantly higher ($P<0.01$) rates of survival than did respective C57 mice. The 47.4% difference between BALB P gen experimentals (81.4%) and C57 F1 gen experimentals (33.7%) was the greatest interstrain difference in survivorship. The fact that BALB mice are larger than C57 mice and that there is a 22-25% difference in survivorship among controls are interstrain variations unrelated to smoke exposure.

Although there were no significant intrastrain or interstrain differences in sex ratios, female pups of both strains tended to have a higher survival rate than males (Table 2). The only indication that one sex might be negatively affected more than the other is shown by the large decrease in survivorship of P gen experimental females of both strains. This is particularly evident for C57 P gen experimental females, which declined by 14.5% (68.6% to 44.1%) while corresponding males declined by only 7% (59.2% to 52.3%).

The effects of sidestream smoke on perinatal and postnatal mortality are recorded in Table 3. Perinatal mortality represents the dead mice on postnatal day 1 (within 16 hr of parturition). These mice include stillbirths and neonatal deaths, and were

TABLE 3. Effects of sidestream smoke on perinatal (D1) and postnatal (D2-D21) mortality[1].

Strain	Gen	Group	N	Mortality	
				Perinatal (%)	Postnatal (%)
BALB/c	P	Con	28	1/202 (0.5)	21/201 (10.5)
		Exp	26	10/146 (6.9)	25/111 (22.5)
	F1	Con	36	1/263 (0.4)	27/235 (11.5)
		Exp	29	20/197 (10.2)	16/177 (9.0)
C57BL	P	Con	63	3/463 (0.7)	165/460 (35.9)
		Exp	27	3/170 (1.8)	85/167 (50.9)
	F1	Con	30	1/221 (0.5)	74/220 (33.6)
		Exp	29	29/181 (16.0)	91/512 (59.9)

1. Postnatal mortality is significantly higher ($P<0.01$) than perinatal mortality in all cases except that no signif-cant difference occurs between BALB experimetnal F1 gen peri- and postnatal values.

included in the litter size counts. Postnatal mortality represents those mice that died from postnatal day 2 to postnatal day 21 (weaning). Postnatal mortality is significantly higher than perinatal mortality in all but one case. BALB experimental F1 gen mice showed no difference between early and late death.

A decrease in food intake by smoke-exposed dams resulting in undernutrition is a potential variable that could interfere with the results of this study. However, no discernible differences between control and experimental dams were observed regarding their food intake or state of nutrition.

Discussion

More than 2000 compounds have been identified in cigarette smoke, many of which are established carcinogens, irritants, and asphyxiants (49). Currently, the most

widely accepted hypothesis relating maternal smoking and effects on the fetus and newborn is that smoking affects the fetus by way of intrauterine hypoxia. Such hypoxia could occur as a result of one or a number of factors associated with smoking (1).

Carbon monoxide and nicotine are the two substances that have received the most attention (42). Both carbon monoxide and nicotine that women absorb from cigarettes reduce the delivery of oxygen to fetal tissues (28). In the blood, carbon monoxide has a higher affinity for hemoglobin than does oxygen, and it combines with hemoglobin to form carboxyhemoglobin (COHb), thereby producing as much as a 12% reduction of the oxygen-carrying capacity of the blood, resulting in fetal hypoxia (1).

COHb levels in pregnant nonsmokers vary from 0.4 to 4.4%, whereas in chronic smokers, levels may very from 2 to 14%. At birth, these levels in infants of nonsmokers are 0.2 to 3.6% compared to 1.1 to 9.2% for infants of smokers and are close to those which have independently been shown to reduce birth weights, increase perinatal mortality and adversely affect subsequent behavior in animals (1).

It has also been contended that nicotine causes fetal hypoxia by vasoconstriction of uterine blood vessels (40) and decreased perfusion of intervillous spaces (28). The umbilical, placental, and fetal circulation could also be affected by catecholamine release induced by nicotine crossing the placental barrier (12).

The chemical composition of sidestream smoke (smoke emitted into the environment by a smoker between puffs) is qualitatively similar to the mainstream smoke inhaled by the smoker (36). Analytical studies indicate that sidestream smoke contains a larger proportion than mainstream smoke of many of the compounds present in smoke on a per cigarette basis (44). Among the gas phase constituents of cigarette smoke that result in high sidestream (SS)/mainstream (MS) ratios are carbon monoxide (2.5), carbon dioxide (8.1), and ammonia (73.0). Two important particulate phase components with high SS/MS ratio are nicotine (2.7), and "tar" (1.7) (23).

In recent studies, passive smoking (inhalation of sidestream smoke) has been associated with many of the same outcomes as direct smoking (inhalation of mainstream smoke) such as low birth weight (31), aggravation of angina pectoris (4), lung cancer (21, 31), asthma (20), and other respiratory illnesses in children and infants (57, 53, 45). Animal studies have shown that sidestream smoke can result in a decrease in both cell viability and protein synthesis as well as the production of cell stress/heat shock-like polypeptides (24).

Exposure of pregnant animals to concentrations of carbon monoxide that produces COHb values of 11 to 28% lowers birth weight (37), decreases litter size (13), and increases perinatal mortality (30). A mean COHb concentration of 28.3% (BALB) and 30.7% (C57) immediately following smoke exposure under the conditions of this investigation barely exceed the upper end of this range. These concentrations are also consistent with those reported previously for a different mouse strain (33).

The fact that we observed a higher incidence of postnatal mortality than perinatal mortality might indicate that the cause of pup death is due to factors other than, or in addition to, in utero effects of smoke exposure. One possibility is that smoke inhibits lactation, which would effect pup growth and development. Injection of nicotine (7) and inhalation of tobacco smoke (17) inhibits prolactin secretion in rats. Andersen, et al. (3) showed that pregnant women who smoked had significantly lower levels of serum prolactin than nonsmoking pregnant women; whereas, Counsilman and Mackay (11) found that smokers tended to wean earlier than nonsmokers, and suggested that this was caused by tobacco smoke inhibition of milk production. However, since most of our pup mortality occurred early in the postnatal period (between D2 and D5), it appears that in utero effects are probably more significant

to pup mortality than decreased lactation, but further research is needed to substantiate this hypothesis.

A number of studies have shown that humans exposed to sidestream smoke exhibit lower COHb levels than were produced in our study. Russell, et al. (43) found that COHb levels increased from 1.6% to 2.6% in nonsmokers present in a smoke-polluted room with a carbon monoxide level of 38 ppm. Aronow (4) reported that exposure of 10 patients with coronary artery disease to the smoke from 15 cigarettes over a 2 hour period in a 30.8 m^3 room increased the COHb levels of nonsmokers from a baseline of 1.26% to 2.28% when the ventilation was turned off. However, carbon monoxide concentrations as high as 200 ppm in a closed environment have been reported (46). Carbon monoxide levels of 150 ppm and 300 ppm resulted in COHb concentrations of 18.5% and 26.8%, respectively, in rats (52).

Comparison of animal smoke-exposure studies is often difficult to make. There are several reasons for this. Differences in smoking procedures and the kinds of animals used account for much of the difficulty. For example, baseline levels of COHb, which is a major biochemical marker of smoke exposure, can vary significantly among different species. This in turn affects COHb levels produced upon exposure to smoke. One study on the effects of passive smoke exposure on sheep reported COHb baseline concentrations of 6.6% (50). This value compares to baseline COHb averages of 2.2% and 3.8% for the BALB and C57 mice used in this investigation. The differences (8-12 times) between baseline and experimental COHb levels attained in our study approximate those reported for heavy smokers (1). Although the mice in this investigation were exposed to somewhat higher concentrations of sidestream smoke than humans are in a smokefilled environment, the data support the growing number of studies that demonstrate the potential health hazards of sidestream smoke (19, 51). In addition, we have shown that animals of the same species, with similar COHb baseline values, can vary in their susceptibility to sidestream smoke. The strain variation to the sidestream smoke effects observed in this study does not appear to be caused by differences in smoke absorption since both the BALB and C57 mice had similar blood carboxyhemoglobin concentrations following the 40 min smoke exposure. In general, the effects of smoke exposure are more pronounced in the C57 strain. This may be due, in part, to the fact that the C57 mice are about 22% (0.3g) lighter than the BALB mice. However, it would seem that other factors are also involved. In spite of the size differential, C57 controls and BALB controls produced similar litter sizes (7.2-7.4) and sex ratios. In addition, pup weight was generally unaffected by smoke exposure in either strain. A 5.1% decline in the BALB P gen experimental group was the lone exception. A reduction in birth weight (31) would appear to be the most incriminating evidence to date that passive smoking has an affect on human reproduction and development.

Summary

Our results indicate that sidestream smoke which produces COHb concentrations of 28-31% can produce deleterious effects on mouse reproduction and development. They also demonstrate that not all strains of a given type of animal are affected by sidestream smoke equally. We suggest that strain selection is an important variable and should be taken into consideration in future smoke-exposure studies. In conclusion, our results substantiate a recent study of the National Academy of Sciences (10) which stated in its report on indoor pollutants that: "Public policy should clearly articulate that involuntary exposure to tobacco smoke ought to be minimized or avoided where possible."

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The Vertebrate Collection of the Indiana State Museum: "Old" Specimens and Records

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Abstract

Compilation of all "old" (pre-1932) catalogue records, and a recataloguing of all vertebrates in the collection of the Indiana State Museum has resulted in the location of many important specimens and interesting records. Many of the specimens have been lost, however, and many of those existing lack data.

There are 301 entries for Indiana birds, now represented by 90 documented specimens. Included are such important specimens as an American white pelican (Fountain County, 1896), brown pelican (Marion County, 1907), tundra swan (Newton County, 1897), greater prairie chicken (Benton and Fountain counties), passenger pigeon (Wayne County, 1869), and thick-billed murre (Marion County, 1896).

Thirty-eight Indiana mammal records are now represented by eleven specimens. One hundred and seventeen turtle records, most collected by W.S. Blatchley in 1901, are represented by 80 documented specimens. Records exist for 62 additional reptiles, and for twenty-one fishes that are no longer in the collection.

Introduction

Little has been published describing the natural history collections of the Indiana State Museum (INSM). Thompson (27) published a list of invertebrate fossils, samples of building stone, and of freshwater bivalves, the predominant specimens in the collection at that time. Butler (6) referred to several birds in the State Museum collection. Hahn (9) noted that "The State Museum at Indianapolis contains a fairly representative mounted collection, but lacks a number of species of mice, shrews and bats, and has no reserve material for study." Twenty-seven years later Lyon (17), in referring to Indiana mammal collections, relates "A few are recorded by Hahn as in the State Museum. These have been moved about in the State House so many times that their labels have become lost and they are of little scientific value. Unfortunately I have not had the opportunity of assembling it in one place and studying it as a whole." The collections received poor management from the 1920's until 1967 when they were transferred to the present structure (25). From at least the late 1950's through 1981 the natural history collections and their records were generally inaccessible for study (letters, A.S. Horowitz to R.L. Richards, 13 April 1983 and to W. Bruggen, 5 July 1983; R.E. Mumford, pers. comm. October 1987). Reorganization of the natural history collections has been underway since 1982 (26). Richards (25) presented a short history of the collections and a description of the Pleistocene vertebrates.

Because such older works as Butler (6) and Hahn (9) and contemporary publications as Mumford and Keller (21) refer to, or make secondary citations to specimens in the State Museum collections, I thought it timely to make the content of the vertebrate collection known. Because of the sparsity of "early" published records, I am presenting the entire list of INSM specimens and records accumulated from 1877 through 1932.

Methods

I have compiled all "old" records, including labels, miscellaneous records, photographs and a 1932 catalogue list which supplied most of the information. A

cursory review of the literature added some documentation to several specimens. All materials on non-permanent loan were recalled. Existing vertebrates were renumbered and a new catalogue developed. Matching specimens with old catalogue records was often difficult, since three different numbering systems (often overlapping) had been used. Often, one-of-a-kind specimens without numbers could be referred to a unique catalogue listing. Fortunately, most record listings contained common and scientific names, adding to the credibility of identification of specimens that could not be located. Extensive revision to current taxonomic nomenclature was necessary. I did not include in this report records of specimens collected since 1932 nor a collection of several hundred birds eggs. These will be addressed at a later date.

Results

In all, 894 vertebrates were listed in the Museum's records. Many of those specimens have been lost and many of those existing lack data. I have listed both documented specimens (with catalogue numbers), as well as records that are unsupported by specimens (Table 1). Some exotic items, such as a three-toed sloth and great bustard were found to be on permanent loan to The Children's Museum (Indianapolis). Many specimens on loan to schools and other institutions were stolen or mutilated and some were destroyed by dermestids. Some may have been destroyed by dermestids while in the museum.

The fish catalogue consisted of only 47 entries, including such exotic items as sawfish rostra, dried cow fish and burr fish. Of thirty listings for fish found in Indiana, data was available for only 20 specimens, all but two of common occurrence within their modern ranges. There is record of a large blue catfish (*Ictalurus furcatus*) from the East Fork, White River, Lawrence County. If unstocked, this is an unusual occurrence from its normal Ohio River distribution in Indiana. All other fishes in the collection were apparently liquid preserved (alcohol or formaldehyde) and were lost prior to the 1966 move of specimens to the present quarters.

The amphibians and reptiles in the collection also were largely liquid preserved and survive today only as "stuffed" turtles and their dried shells. There are listings for 32 amphibians, 29 with Indiana documentation. The blue-spotted salamander (*Ambystoma laterale*, Starke County) record is among the few for northwestern Indiana. The record for the small-mouthed salamander (*A. texanum*, Starke County) is interesting since Minton (18) has an unconfirmed record for the Jasper-Pulaski County area to the south and for Marshall County to the east. The entry for pickerel frog (*Rana palustris*, Posey County), without specimen, is so displaced west of its modern range that I question its identification.

There are 103 entries for reptiles (exclusive of turtles), including alligators, iguanas, rattlesnake rattles and various snake eggs. One unusual Indiana listing, 24 June 1931 notes: "Blunt-head snake; shipped from South America to Indianapolis in a carload of bananas; mildly poisonous; 30 inches in length; Donated by F. Venizia, Indianapolis." Seventy-nine listings of Indiana occurring species produced data on 27 snake and 6 lizard specimens. A listing for the midland brown snake (*Storeria dekayi*), Dubois County, is curious, since this species is poorly documented in southwestern Indiana, with only an unconfirmed record for Posey County (18). The few records for the Plains garter snake (*Thamnophis radix*), include reports from northwestern Indiana and the only listing for LaPorte County. The listing of the red milk snake (*Lampropeltis triangulum syspila*) for Posey County is noteworthy, as Minton (18) also has an unconfirmed record for that county, the only record in extreme southwestern Indiana. The "red" snake is clearly separated from the two other listed milk snake specimens in the Museum's catalogue. An unusual "two headed" eastern hognose snake (*Heterodon platyrhinos*) was found near Manilla, Rush County.

TABLE 1. Documented Indiana Vertebrate Specimens and Records, "Old" Collection (pre-1932), Indiana State Museum.[1]

Cat #/Rec.	Name	Locality	Date	Comments
R	*Ichthyomyzon unicuspis*, silver lamprey	Marion Co.; White R.	pre-1932	
R	*Dorosoma cepedianum*, gizzard shad	Knox Co.; Wabash R.	pre-1932	
R	*Esox americanus vermiculatus*, grass pickerel	Marion Co.; Fall Cr.	pre-1932	
R	*Esox americanus vermiculatus*, grass pickerel	Noble Co.; Rome City, Elkhart, R.	pre-1932	2 specimens
R	*Esox americanus vermiculatus*, grass pickerel	Starke Co.; Bass Lake	pre-1932	
R	*Esox americanus vermiculatus*, grass pickerel	Tippecanoe R. marshland	pre-1932	
R	*Notemigonus chrysoleucas*, golden shiner	LaPorte Co.; Clear Lake	pre-1932	
R	*Notropis whipplei*, steelcolor shiner	Owen Co.; Eel R.	pre-1932	
R	*Catostomus commersonii*, white sucker	Noble Co.; Rome City	pre-1932	
R	*Ictalurus natalis*, yellow bullhead	Putnam Co.; Walnut Cr.	pre-1932	2 specimens
R	*Ictalurus nebulosus*, brown bullhead	Marion Co.; Fall Cr.	pre-1932	
R	*Ictalurus nebulosus*, brown bullhead	Starke Co.; Bass Lake	pre-1932	
R	*Ictalurus furcatus*, blue catfish	Lawrence Co.; East Fork, White R., Williams Dam	caught by Pluggie Niman; purchased 5 Nov. 1926 by Dept of J.B. Welborn; 4′4½″, 78 lbs.	
R	*Micropterus salmoides*, largemouth bass	LaPorte Co.; Pine Lake	pre-1932	
R	*Micropterus salmoides*, largemouth bass	Fulton Co.; Manitou Lake	pre-1932	
R	*Lepomis macrochirus*, bluegill	LaPorte Co.; Pine Lake	pre-1932	
R	*Lepomis megalotis*, longear sunfish	Marion Co.; Fall Cr.	pre-1932	
R	*Amboplites rupestris*, rock bass	Tippecanoe R. marshland	pre-1932	
R	*Pomoxis nigromaculatus*, black crappie	LaPorte Co.; Pine Lake	pre-1932	
R	*Ambystoma laterale*, blue-spotted salamander	Starke, Co.; Bass Lake	pre-1932	
R	*Ambystoma maculatum*, spotted salamander	Lawrence Co.; Mitchell	pre-1932	
R	*Ambystoma maculatum*, spotted salamander	"Vawter Park"	3 June 1902	
R	*Ambystoma tigrinum*, tiger salamander	Vigo Co.	pre-1932	2 specimens
R	*Ambystoma tigrinum*, tiger salamander	Starke Co.; Bass Lake	27 July 1902	
R	*Ambystoma tigrinum*, tiger salamander	Lawrence Co.; Mitchell	pre-1932	
R	*Ambystoma opacum*, marbled salamander	Knox Co.	3 May 1902	

[1] abbreviations used: Cat #, present catalogue number; R, record only (no specimen); D, destroyed (specimen badly deteriorated); M, male; F, female. Note: reference is to Indiana localities, unless otherwise noted. Literature citation in brackets ( ) under comments. Localities and dates in brackets ( ) refer to information constucted from the literature, and not present in the Museum records.

TABLE 1.—Continued

Cat #/Rec.	Name	Locality	Date	Comments
R	*Ambystoma opacum*, marbled salamander	Knox Co.; Cypress Swamp	pre-1932	
R	*Ambystoma texanum*, small-mouth salamander	Knox Co.; Cypress Swamp	pre-1932	
R	*Ambystoma texanum*, small-mouth salamander	Starke Co.; Bass Lake	23 Aug. 1902	
R	*Plethodon glutinosus*, slimy salamander	Perry Co.; Cannelton	1 May 1902	2 specimens
R	*Plethodon cinereus*, redbacked salamander	Franklin Co.; Laurel	16 May 1902	
R	*Plethodon cinereus*, redbacked salamander	Putman Co.	9 Nov. 1902	
R	*Plethodon cinereus*, redbacked salamander	Perry Co.; Cannelton	pre-1932	
R	*Eurycea bislineata*, two-lined salamander	Putman Co.	9 Nov. 1902	5 specimens
R	*Eurycea lucifuga*, cave salamander	Crawford Co.; Wyandotte Cave	25 May 1902	
R	*Eurycea lucifuga*, cave salamander	Crawford Co.; Wyandotte Cave	pre-1932	
R	*Eurycea longicauda*, long-tailed salamander	Franklin Co.; Laurel	16 May 1902	
R	*Siren intermedia*, lesser siren	Starke Co.; Bass Lake	10 May 1900	2 specimens
R	*Rana pipiens* complex, leopard frog	"Vawter Park"	11 Aug. 1902	
R	*Rana pipiens* complex, leopard frog	Crawford Co.; Wyandotte	pre-1932	
R	*Rana palustris*, pickerel frog	Posey Co.; Grand Chain	pre-1932	
R	*Chelydra serpentina*, common snapping turtle	Wabash Co., IL; Mt. Carmel	pre-1932	
R	*Chelydra serpentina*, common snapping turtle	Wayne Co.; Washington Twp., McGrew's pond.	Max Munte, Cambridge City, Sept. 1901; 21½ lbs.	
71.7.6	*Macroclemys temmincki*, alligator snapping turtle	"Arkansas"	skull donated March 1877 (10) by Elias Wilson, Indpls.	
R	*Sternotherus odoratus*, musk turtle	Starke Co.; Bass Lake	pre-1932	2 specimens
R	*Sternotherus odoratus*, musk turtle	Wabash Co., IL; Mt. Carmel	pre-1932	
71.7.4	*Kinosternon subrubrum*, mud turtle	Starke Co.; Bass Lake	May 1902	
71.7.7, and R	*Clemmys guttata*, spotted turtle	Starke Co.; Bass Lake	pre-1932	2 records, one existing specimen
R	*Emydoidea blandingii*, Blanding's turtle	Starke Co.; Bass Lake	May 1901	2 specimens
R	*Emydoidea blandingii*, Blanding's turtle	Lake Co.; Hammond	1 Sept. 1902	
R	*Terrapene carolina*, Eastern box turtle	Posey Co.; New Harmony	May 1902	
R	*Terrapene carolina*, Eastern box turtle	Posey Co.; New Harmony	Oct. 1901	3 specimens
71.7.5.8	*Terrapene ornata*, ornate box turtle	Clinton, MO	July 1901	
R	*Terrapene ornata*, ornate box turtle	Posey Co.	pre-1932	
71.7.1.18	*Graptemys geographica*, map turtle	Posey Co.; Grand Chain, Wabash R.	Aug. 1901	

71.7.2.8, .11, .13	*Graptemys geographica*, map turtle	Wabash Co., IL; Mt. Carmel	July 1901	
71.7.3.21, .22, .31, .38	*Graptemys geographica*, map turtle	Posey Co.; Grand Chain and Wabash Co., IL; Mt. Carmel	1901-1902	
R	*Graptemys pseudogeographica*, midland sawback turtle	Posey Co.; Grand Chain	1901-1902	
71.7.3.18 – .20, .37, .40	*Graptemys pseudogeographica*, midland sawback turtle	Posey Co.; Grand Chain and Wabash Co., IL; Mt. Carmel	1901-1902	
R	*Graptemys* sp., map/sawback turtle	Wabash Co., IL; Mt. Carmel	1901-1902	2 specimens
R	*Chrysemys picta marginata*, midland painted turtle	Wabash Co., IL; Mt. Carmel	1901-1902	3 specimens
71.7.1.41	*Chrysemys picta*, midland painted turtle	Posey Co.; Grand Chain, Wabash R.	Aug. 1901	
71.7.1.1 – .17; .19 – .40	*Pseudemys scripta elegans*, red-eared turtle	Posey Co.; Grand Chain, Wabash R.	1901 (coll. two in July, 35 in Aug., two in Oct.)	
71.7.2.1, .3, .7, .9, .10, .12	*Pseudemys scripta elegans*, red-eared turtle	Wabash Co., IL; Mt. Carmel	July 1901 when listed	
71.7.3.1 – .17	*Pseudemys scripta elegans*, red-eared turtle	Posey Co.; Grand Chain, and Wabash Co., IL; Mt. Carmel	unmarked specimens apply to 24 Grand Chain records (most Aug., two Oct. 1901), and 9 Mt. Carmel records (July 1901)	
R	*Pseudemys scripta elegans*, red-eared turtle	Marshall Co.; Lake Maxinkuckee	pre-1932	John Peckham, Indpls.
R	*Pseudemys concinna hieroglyphica* × *P. floridana hoyi*, hieroglyphic turtle	Posey Co.; Grand Chain, Wabash R.	1901-1902 (one coll. Aug. 1901)	3 specimens
71.7.2.2	*Pseudemys concinna hieroglyphica* × *P. floridana hoyi*, hieroglyphic turtle	Wabash Co., IL; Mt. Carmel	1901-1902	
71.7.3.26, .34-.36	*Pseudemys concinna hieroglyphica* × *P. floridana hoyi*, hieroglyphic turtle	Posey Co.; Grand Chain, Wabash R. applies to 3 specimens.	1901-1902	
R	*Sceloporus undulatus*, fence liazard	Perry Co.; Cannelton	8 May 1901	
R	*Ophisaurus attenuatus*, western ophisaur	Lake Co.; Hammond	19 Aug. 1902	
R	*Cnemidophorus sexlineatus*, sixlined racerunner	Lake Co.; Pine	25 July 1902	
R	*Eumeces fasciatus*, five-lined skink	Lawrence Co.; Mitchell	17 July 1902	
R	*Eumeces fasciatus*, five-lined skink	Perry Co.; Cannelton	8 May 1901	
R	*Eumeces fasciatus*, five-lined skink	Crawford Co.	26 June 1902	
R	*Nerodia sipedon*, banded watersnake	Lake Co.; Shelby	pre-1932	

TABLE 1.—Continued

Cat #/Rec.	Name	Locality	Date	Comments
R	*Clonophis kirtlandii*, Kirtland's snake	?Steuben Co.; James Lake	pre-1932	2 specimens
R	*Storeria dekayi*, midland brown snake	Dubois Co.; Huntingburg	pre-1932	
R	*Storeria dekayi*, midland brown snake	Lake Co.	pre-1932	
R	*Thamnophis radix*, plains garter snake	LaPorte Co.; Wilders	19 May 1902	
R	*Thamnophis radix*, plains garter snake	Lake Co.; Shelby	pre-1932	
R	*Thamnophis radix*, plains garter snake	Lake Co.; Hammond	24 July 1902	
R	*Coluber constrictor flaviventris*, blue racer	Starke Co.; Bass Lake	24 Aug. 1902	
R	*Elaphe obsoleta*, black rat snake	Posey Co.; New Harmony	6 May 1902	
R	*Elaphe obsoleta*, black rat snake	Posey Co.; New Harmony	May 1901	
R	*Elaphe vulpina*, fox snake	Starke Co.; Bass Lake	pre-1932	
R	*Lampropeltis getulus*, black kingsnake	Posey Co.	pre-1932	
R	*Lampropeltis triangulum syspila*, red milk snake	Posey Co.	6 May 1902	
R	*Lampropeltis triangulum triangulum*, eastern milk snake	Kosciusko Co.	6 April and 4 May 1901	2 specimens
R	*Opheodrys aestivus*, rough green snake	Crawford Co.; Wyandotte	4 Sept. 1902	
R	*Opheodrys aestivus*, rough green snake	Crawford Co.	pre-1932	
R	*Diadophis punctatus*, ringneck snake	Franklin Co.; Laurel	16 May 1902	
R	*Diadophis punctatus*, ringneck snake	Lawrence Co.	21 May 1901	
R	*Carphophis amoenus*, worm snake	Crawford Co.; Wayndotte	pre-1932	
R	*Carphophis amoenus*, worm snake	Posey Co.	pre-1932	
R	*Heterodon platyrhinos*, eastern hognose snake	Lake Co.; Pine	25 July 1902	
R	*Heterodon platyrhinos*, eastern hognose snake	Posey Co.; New Harmony	4 May 1901	
R	*Heterodon platyrhinos*, eastern hognose snake	Rush Co.; Manilla	pre-1932	2 headed; S.C. Peck
R	*Heterodon platyrhinos*, eastern hognose snake	Morgan Co.; near Martinsville	22 Sept. 1925	Mrs. Kramer, Indpls.
R	*Sistrurus catenatus*, eastern massasauga	LaPorte Co.; LaPorte	pre-1932	
71.8.2	*Gavia immer*, common loon	Boone Co.	pre-1932	
71.8.3	*Gavia immer*, common loon	Brown Co.	pre-1932	
71.8.4	*Gavia immer*, common loon	Brown Co.	pre-1932	
71.8.5	*Gavia immer*, common loon	Marion Co.; Broad Ripple	1902	M; W.D. Cost, Indpls.
R	*Gavia immer*, common loon	Marion Co.; Broad Ripple	1902	M; W.D. Cost, Indpls.

71.8.15	*Podilymbus podiceps*, pied-billed grebe	Boone Co.	pre-1932	M
71.8.10	*Podiceps auritus*, horned grebe	Starke Co.; Bass Lake	27 Apr. 1903	M; Frank Kelley
R	*Podiceps auritus*, horned grebe	Starke Co.; Bass Lake	27 Apr. 1903	M; Frank Kelley
71.8.24	*Pelecanus erythrorhynchos*, American white pelican	Fountain Co.; Attica	20 Apr. 1896	head separated (4)
71.8.25	*Pelecanus occidentalis*, brown pelican	Marion Co.; near Broad Ripple	26 Mar. 1907	M; W.K. Dean (4,11,21)
71.8.26	*Phalacrocorax auritus*, double-crested comorant	Hamilton Co.	Oct. 1900	M; imm.
71.8.27	*Phalacrocorax auritus*, double-crested comorant	?Bartholomew Co.	pre-1932	J.B. Crum
71.8.36	*Botaurus lentiginosus*, American bittern	Boone Co.	pre-1932	M
D	*Botaurus lentiginosus*, American bittern	Boone Co.	pre-1932	M
71.8.34	*Ixobrychus exilis*, least bittern	Boone Co.	pre-1932	F; dismounted (6)
R	*Ixobrychus exilis*, least bittern	Boone Co.	pre-1932	M (6)
R	*Ardea herodias*, great blue heron	Brown Co.	1896	M and F
R	*Ardea herodias*, great blue heron	Morgan Co.; near Waverly	1890	M; Fred Scruggs, Mooresville
71.8.30	*Egretta caerulea*, little blue heron	Hamilton Co.	pre-1927	Prof. Irvin Stanley, 8 March 1927
R	*Egretta caerulea*, little blue heron	Putman Co.; near Bainbridge	pre-1932	M; John Michaels
R	*Butorides striatus*, green-backed heron	Boone Co.	pre-1932	two M, one F
D	*Nycticorax nycticorax*, black-crowned night-heron	Boone Co.	pre-1932	M; imm.
71.8.33	*Nycticorax violaceus*, yellow-crowned night-heron	?Boone Co.	1927	written on base: "mounted by Elmer S. Pratt, Lebanon, Ind. 1927"
71.8.40	*Cygnus columbianus*, tundra swan	Netwon Co.; near Moracco	14 Oct. 1897	M (6)
71.8.52	*Chen caerulescens*, snow goose	Porter Co.	23 Mar. 1898	F; barred pattern
71.8.41	*Branta canadensis*, Canada goose	Porter Co.	12 Apr. 1898	M
R	*Branta canadensis*, Canada goose	Porter Co.	12 Apr. 1898	F
71.8.102	*Aix sponsa*, wood duck	Boone Co.	pre-1932	M
D	*Aix sponsa*, wood duck	Boone Co.	pre-1932	F
R	*Anas platyrhynchos*, mallard	Boone Co.	pre-1932	M and F
71.8.78	*Anas acuta*, northern pintail	Porter Co.	pre-1932	M
71.8.79	*Anas acuta*, northern pintail	Porter Co.	pre-1932	F
R	*Anas discors*, blue-winged teal	Boone Co.	pre-1932	M
71.8.98	*Anas clypeata*, northern shoveler	Porter Co.	pre-1932	F
R	*Anas clypeata*, northern shoveler	Boone Co.	pre-1932	M
71.8.112	*Aythya collaris*, ring-necked duck	Porter Co.	pre-1932	M
71.8.113	*Aythya collaris*, ring-necked duck	Porter Co.	pre-1932	F
R	*Aythya affinis*, lesser scaup	Boone Co.	pre-1932	M and F

TABLE 1.—Continued

Cat #/Rec.	Name	Locality	Date	Comments
71.8.129	*Clangula hyemalis*, oldsquaw	Boone Co.	pre-1932	F
D and R	*Bucephala albeola*, bufflehead	Boone Co.	pre-1932	M (D) and F (R)
R	*Lophodytes cucullatus*, hooded merganser	Boone Co.	pre-1932	F
R	*Mergus serrator*, red-breasted merganser	Ripley Co.	pre-1932	M
71.8.637	*Cathartes aura*, turkey vulture	Brown Co.	20 June 1898	
71.8.168	*Pandion haliaetus*, osprey	Putman Co.	30 Apr. 1904	John Michaels
R	*Pandion haliaetus*, osprey	Bartholomew Co.	20 June 1927	Don. through A. W. Butler by county hospital (Columbus), where it was taken.
71.8.160	*Haliaeetus leucocephalus*, bald eagle	Putman Co.	pre-1932	M; juv.; John Michaels, Bainbridge
R	*Accipiter cooperi*, Cooper's hawk	Boone Co.	pre-1932	M
R and D	*Buteo jamaicensis*, red-tailed hawk	Boone Co.	pre-1932	M (R) and F (D)
R	*Buteo jamaicensis*, red-tailed hawk	Morgan Co.	pre-1932	two M (one by A.J. Coleman)
R	*Buteo jamaicensis*, red-tailed hawk	Morgan Co.	pre-1932	F; A.J. Coleman
R	?*Buteo regalis*, ferruginous hawk	Brown Co.	10 May 1894	
71.8.641 and R	*Buteo lagopus*, rough-legged hawk	Brown Co.	pre-1932	F and M (R)
71.8.156	*Aquila chrysaetos*, golden eagle	Shelby Co.; Jackson township	pre-1932	O.H. Williams, Flat Rock
R	*Falco sparverius*, American kestrel	Boone Co.	pre-1932	2 specimens
71.8.171	*Falco columbarius*, merlin	Boone Co.	3 Mar. 1874	(6)
71.8.169	*Falco peregrinus*, peregrine falcon	(Boone Co.; Slabtown)	(14 May 1896)	listed as M marsh hawk (6)
R	*Gennaeus nycthemerus*, silver pheasant	State Game Reserve, Waveland	pre-1932	
R	*Chrysolophus pictus*, golden pheasant	State Game Reserve, Waveland	pre-1932	
R	*Bonasa umbellus*, ruffed grouse	Boone Co.	pre-1932	M and F
71.8.209	*Tympanuchus cupido*, greater prairie-chicken	Fountain Co.	pre-1932	written on base: "*Tympanuchus americanus*, Fountain County, W.L. Wood"
71.8.211	*Tympanuchus cupido*, greater prairie-chicken	Benton Co.	pre-1898	M (6)
R	*Tympanuchus cupido*, greater prairie-chicken	Benton Co.	pre-1932	F; possibly 71.8.210

71.8.219	*Meleagris gallopavo*, wild turkey	Dirk, MO	14 Dec. 1909	M
71.8.220	*Meleagris gallopavo*, wild turkey	Dirk, MO	14 Dec. 1909	M
R	*Colinus virginianus*, northern bobwhite	Boone Co.	pre-1932	"family of quail"
71.8.192	*Colinus virginianus texanus*, Texas bobwhite	State Game Reserve, Waveland	8 Jan. 1920	M
R	*Colinus virginianus texanus*, Texas bobwhite	State Game Reserve, Waveland	8 Jan. 1920	F
71.8.230	*Rallus elegans*, king rail	Boone Co.	pre-1932	
R	*Rallus elegans*, king rail	Boone Co.	pre-1932	
71.8.233	*Rallus limicola*, Virginia rail	Boone Co.	3-9 May 1895	M; dismounted
R	*Rallus limicola*, Virginia rail	Marion Co.	Aug. 1898	F
71.8.235	*Porzana carolina*, sora	Henry Co.; Middleton	2 May 1908	partial albino
71.8.236	*Porzana carolina*, sora	Vigo Co.	pre-1932	F
R	*Porzana carolina*, sora	Vigo Co.	19 May 1893	M
71.8.238	*Gallinula chloropus*, common moorhen	Boone Co.	pre-1932	M
71.8.239	*Gallinula chloropus*, common moorhen	Boone Co.	pre-1932	F
71.8.240	*Gallinula chloropus*, common moorhen	Boone Co.	pre-1932	F
71.8.242	*Fulica americana*, American coot	Brown Co.	pre-1932	M
71.8.243	*Fulica americana*, American coot	Boone Co.	pre-1932	F
R	*Grus canadensis*, sandhill crane	Montgomery Co.; near Darlington	16 Apr. 1900	
71.8.263	*Pluvialis dominica*, lesser golden plover	Benton Co.	pre-1932	M
R	*Pluvialis dominica*, lesser golden plover	?Benton Co.	pre-1932	two M, two F
R	*Charadrius semipalmatus*, semipalmated plover	Jasper Co.	pre-1932	M (6)
R	*Charadrius vociferous*, killdeer	Boone Co.	6 May 1895	M
71.8.258	*Tringa solitaria*, solitary sandpiper	Boone Co.	pre-1932	M
R	*Tringa solitaria*, solitary sandpiper	Boone Co.	pre-1932	F
R	*Actitis macularia*, spotted sandpiper	Boone Co.	pre-1932	M
71.8.248	*Limosa fedoa*, marbled godwit	LaPorte Co.	pre-1932	F
R	*Limosa fedoa*, marbled godwit	LaPorte Co.	pre-1932	M
R	*Calidris minutilla*, least sandpiper	Vigo Co.	19 May 1888	M
R	*Calidris melanotos*, pectoral sandpiper	Vigo Co.	21 Apr. 1888	M and F
R	*Gallinago gallinago*, common snipe	Monroe Co.	25 Mar. 1886	M
R	*Scolopax minor*, American woodcock	Boone Co.	pre-1932	F
71.8.262	*Phalaropus tricolor*, Wilson's phalarope	(Boone Co.; Lebanon)	(9 May 1889)	M (also F rec.) (6)
71.8.261	*Phalaropus lobatus*, red-necked phalarope	(Boone Co.)	(7 June 1889)	M (6,21)
R	*Phalaropus lobatus*, red-necked phalarope	(Boone Co.)	(7 June 1889)	F (6,21)
R	*Phalaropus fulicaria*, red phalarope	Daviess Co.	21 Sept. 1907	F
R	*Larus delawarensis*, ring-billed gull	Boone Co.	pre-1932	F
71.8.272	*Larus argentatus*, herring gull	?Marion Co.; White R., near Belt railroad	Feb. 1899	J.J. Kramer

TABLE 1.—Continued

Cat #/Rec.	Name	Locality	Date	Comments
71.8.279	*Uria lomvia*, thick-billed murre	Marion Co.	13 Dec. 1896	(6,21)
R	*Zenaida macroura*, mourning dove	Boone Co.	pre-1932	M
71.8.286	*Ectopistes migratorius*, passenger pigeon	Wayne Co.	1869	Hon. Walter S. Ratliff, Richmond (13)
R	*Coccyzus erythropthalmus*, black-billed cuckoo	Boone Co.	pre-1932	M
71.8.290	*Coccyzus americanus*, yellow-billed cuckoo	Boone Co.	pre-1932	M
R	*Coccyzus americanus*, yellow-billed cuckoo	Boone Co.	pre-1932	F
71.8.292	*Tyto alba*, common barn owl	Marion Co.	20 Mar. 1896	M
71.8.293	*Tyto alba*, common barn owl	Boone Co.	pre-1932	M
71.8.294	*Tyto alba*, common barn owl	Boone or Johnson Co.	pre-1932	M (6)
R	*Tyto alba*, common barn owl	?Boone Co.	pre-1929	Venna Leonard, Lebanon 9 Sept. 1929
R	*Otus asio*, eastern screech owl	Boone Co.	pre-1932	M and F
R	*Otus asio*, eastern screech owl	Montgomery Co.; State Game Reserve, Waveland	pre-1932	F
71.8.299	*Bubo virginianus*, great horned owl	?Boone Co.	pre-1932	
R	*Bubo virginianus*, great horned owl	?Knox Co.	pre-1932	M, ? and F; B.F. Johnson, Edwardsport
71.8.309	*Nyctea scandiaca*, snowy owl	Mercer/Auglaize, Co., OH; near St. Mary's Reservoir	1880	Mather R. Hull
R	*Nyctea scandiaca*, snowy owl	Montgomery Co.	1 Nov. 1892	M
R	*Strix varia*, barred owl	Brown Co.	pre-1932	M
71.8.317	*Asio otus*, long-eared owl	Boone Co.	pre-1932	M
R	*Asio otus*, long-eared owl	Marion Co.	Dec. 1904	F
71.8.321	*Asio flammeus*, short-eared owl	Boone Co.	pre-1932	F
71.8.680	*Asio flammeus*, short-eared owl	Boone Co.	pre-1932	M
71.8.327	*Aegolius acadicus*, northern saw-whet owl	Boone Co.	pre-1932	M
R	*Chordeiles minor*, common nighthawk	Boone Co.	pre-1932	M
R	*Caprimulgus vociferus*, whip-poor-will	Boone Co.	pre-1932	F
R	*Chaetura pelagica*, shimney swift	Boone Co.	pre-1932	M and F
R	*Archilochus colubris*, ruby-throated hummingbird	Boone Co.	pre-1932	M and F
R	*Ceryle alcyon*, belted kingfisher	Boone Co.	pre-1932	M
R	*Melanerpes erythrocephalus*, red-headed woodpecker	Boone Co.	pre-1932	two M

R	*Melanerpes carolinus*, red-bellied woodpecker	Boone Co.	pre-1932	M and F
R	*Sphyrapicus varius*, yellow-bellied sapsucker	Boone Co.	pre-1932	M and F
R	*Picoides pubescens*, downy woodpecker	Boone Co.	pre-1932	M and F
R	*Picoides villosus*, hairy woodpecker	Boone Co.	pre-1932	M and F
R	*Picoides arcticus*, black-backed woodpecker	Porter Co.; Mineral Springs	21 Nov. 1920	M; H.L. Stoddard
R	*Colaptes auratus*, northern flicker	Boone Co.	pre-1932	M and F
71.8.335	*Dryocopus pileatus*, pileated woodpecker	Fountain Co.	pre-1898	F (6)
R	*Dryocopus pileatus*, pileated woodpecker	Fountain Co.	pre-1898	M (6)
R	*Contopus virens*, eastern wood-pewee	Boone Co.	pre-1932	M
R	*Myiarchus crinitus*, great crested flycatcher	Boone Co.	pre-1932	M
R	*Tyrannus tyrannus*, eastern kingbird	Boone Co.	pre-1932	M
R	*Eremophilia alpestris*, horned lark	Vigo Co.	15 Oct. 1887	M and F
71.8.342	*Progne subis*, purple martin	Boone Co.	pre-1932	M
R	*Progne subis*, purple martin	Boone Co.	pre-1932	F
R	*Stelgidopteryx serripennis*, northern rough-winged swallow	Monroe Co.	11 May 1886	M
71.8.341	*Hirundo rustica*, barn swallow	Boone Co.	pre-1932	M
R	*Cyanocitta cristata*, blue jay	Boone Co.	pre-1932	M
71.8.347	*Corvus brachyrhynchos*, American crow	Greene Co.	pre-1932	albino; James Dixon, Switz City
71.8.644	*Corvus brachyrhynchos*, American crow	Marion Co.	pre-1932	F; albino
R	*Corvus brachyrhynchos*, American crow	Boone Co.	pre-1932	
R	*Parus atricapillus*, black-capped chickadee	Monroe Co.	15 May 1886	M
R	*Parus bicolor*, tufted titmouse	Boone Co.	pre-1932	M
R	*Sitta canadensis*, red-breasted nuthatch	Monroe Co.	pre-1932	M and F
R	*Sitta carolinensis*, white-breasted nuthatch	Boone Co.	pre-1932	M and F
R	*Certhia americana*, brown creeper	Boone Co.	pre-1932	M and F
R	*Troglodytes aedon*, house wren	Vigo Co.	28 Apr. 1888	M
71.8.352	*Troglodytes troglodytes*, winter wren	Monroe Co.	pre-1932	M
R	*Troglodytes troglodytes*, winter wren	Monroe Co.	26 Mar. 1886 and pre-1932	two F
R	*Regulus calendula*, ruby-crowned kinglet	Boone Co.	pre-1932	M
R	*Polioptila caerulea*, blue-gray gnatcatcher	Monroe Co.	12 Apr. 1886	M and F
71.8.361	*Sialia sialis*, eastern bluebird	Boone Co.	pre-1932	M
R	*Sialia sialis*, eastern bluebird	Boone Co.	pre-1932	F
R and D	*Catharus minimus*, gray-cheeked thrush	Monroe Co.	pre-1932	M (R) and F (D)
R	*Catharus ustulatus*, Swainson's thrush	unlisted	8 May 1890	M
R	*Catharus guttatus*, hermit thrush	Boone Co.	pre-1932	M and F
R	*Hylocichla mustelina*, wood thrush	Boone Co.	pre-1932	M

TABLE 1.—Continued

Cat #/Rec.	Name	Locality	Date	Comments
R	*Turdus migratorius*, American robin	Boone Co.	pre-1932	M
71.8.354	*Dumetella carolinensis*, gray catbird	Boone Co.	pre-1932	M
R	*Dumetella carolinensis*, gray catbird	Boone Co.	pre-1932	F
R	*Mimus polyglottos*, northern mockingbird	Boone Co.	pre-1932	M and F
R	*Toxostoma rufum*, brown thrasher	Boone Co.	pre-1932	
R	*Bombycilla garrulus*, Bohemian waxwing	?Porter Co.; Miller's Lake	pre-1932	M; H.L. Stoddard
R	*Bombycilla cedrorum*, cedar waxwing	Boone Co.	pre-1932	M and F
R	*Lanius excubitor*, northern shrike	Monroe Co.	pre-1932	M (21)
R	*Lanius excubitor*, northern shrike	Brown Co.	11 Oct. 1893	F (21)
71.8.368	*Lanius ludovicianus*, loggerhead shrike	unknown	pre-1932	Eugene Buehler, 16 Apr. 1932
R	*Lanius ludovicianus*, loggerhead shrike	Boone Co.	pre-1932	M and F
71.8.372	*Vireo solitarius*, solitary vireo	Boone Co.	pre-1932	M
R	*Vireo gilvus*, warbling vireo	Boone Co.	pre-1932	M and F
R	*Viero olivaceus*, red-eyed vireo	Boone Co.	pre-1932	M
71.8.374 –.375	*Vermivora pinus*, blue-winged warbler	Boone Co.	pre-1932	M and F, same base
R	*Vermivora chrysoptera*, golden-winged warbler	Boone Co.	pre-1932	M
R	*Vermivora chrysoptera*, golden-winged warbler	Franklin Co.	pre-1932	M
D	*Vermivora ruficapilla*, Nashville warbler	Vigo Co.	8 May 1888	M
R	*Dendroica petechia*, yellow warbler	Boone Co.	pre-1932	M and F
R	*Dendroica pensylvanica*, chestnut-sided warbler	Boone Co.	pre-1932	M
71.8.378	*Dendrocia magnolia*, magnolia warbler	Boone Co.	pre-1932	M
R	*Dendrocia magnolia*, magnolia warbler	Vigo Co.	pre-1932	F
R	*Dendroica tigrina*, Cape May warbler	Boone Co.	pre-1932	M
71.8.379	*Dendroica caerulescens*, black-throated blue warbler	Boone Co.	pre-1932	M
R	*Dendroica coronata*, yellow-rumped warbler	Boone Co.	pre-1932	M and F
R	*Dendroica virens*, black-throated green warbler	Boone Co.	pre-1932	M and F
R	*Dendroica fusca*, Blackburnian warbler	Boone Co.	pre-1932	M
71.8.383	*Dendroica pinus*, pine warbler	Warren Co.	pre-1932	M; dismounted
R	*Dendroica discolor*, prairie warbler	Boone Co.	pre-1932	M and F (6)
R	*Dendroica palmarum*, palm warbler	Vigo Co.	21 Apr. 1888	M
D	*Dendroica castanea*, bay-breasted warbler	Boone Co.	pre-1932	M
R	*Dendroica striata*, blackpoll warbler	Boone Co.	pre-1932	M and F
D	*Dendroica cerulea*, Cerulean warbler	Vigo Co.	12 May 1888	M

R	*Mniotilta varia*, black-and-white warbler	Boone Co.	pre-1932	M
R	*Mniotilta varia*, black-and-white warbler	Monroe Co.	1 May 1886	F
R	*Setophaga ruticilla*, American redstart	Boone Co.	pre-1932	M and F
R	*Helmitheros vermivorous*, worm-eating warbler	Monroe Co.	1 May 1886	M
71.8.385	*Seiurus noveboracensis*, northern waterthrush	Boone Co.	pre-1932	M
71.8.386	*Geothlypis trichas*, common yellowthroat	Boone Co.	pre-1932	M
71.8.387	*Geothlypis trichas*, common yellowthroat	Boone Co.	pre-1932	M
R	*Wilsonia citrina*, hooded warbler	Boone Co.	pre-1932	M
71.8.389	*Wilsonia pusilla*, Wilson's warbler	Boone Co.	pre-1898	M; dismounted head missing (6)
D and R	*Wilsonia canadensis*, Canada warbler	Monroe Co.	pre-1932	M (D) and F (R)
R	*Icteria virens*, yellow-breasted chat	Boone Co.	pre-1932	M and F
R	*Piranga rubra*, summer tanager	Vigo Co.	5 May 1888	M
R	*Piranga rubra*, summer tanager	Vigo Co.	29 May 1894	M; imm.
R	*Piranga olivacea*, scarlet tanager	Boone Co.	pre-1932	M and F
R	*Cardinalis cardinalis*, northern cardinal	Boone Co.	pre-1932	M and F
71.8.424	*Pheucticus ludovicianus*, rose-breasted grosbeak	Boone Co.	pre-1932	M
R	*Pheucticus ludovicianus*, rose-breasted grosbeak	Boone Co.	pre-1932	F
71.8.425	*Spiza americana*, dickcissel	Monroe Co.	5 May 1886	M
R	*Pipilo erythrophthalmus*, rufus-sided tohee	Boone Co.	pre-1932	M and F
R	*Spizella arborea*, American tree sparrow	Vigo Co.	25 Nov. 1889	F
R	*Spizella passerina*, chipping sparrow	Boone Co.	pre-1932	M and F
R	*Spizella pusilla*, field sparrow	Marion Co.	13 Apr. 1886	F
71.8.434	*Chondestes grammacus*, lark sparrow	Monroe Co.	pre-1932	M
R	*Passerculus sandwichensis*, savannah sparrow	Starke Co.	pre-1932	F (6)
D	*Ammodramus savannarum*, grasshopper sparrow	Boone Co.	pre-1932	
R	*Ammodramus leconteii*, LeConte's sparrow	Boone Co.	pre-1932	M (6)
71.8.437	*Passerella iliaca*, fox sparrow	Vigo Co.	30 Mar. 1891	F
R	*Passerella iliaca*, fox sparrow	Monroe Co.	25 Mar. 1886	M
R	*Melospiza melodia*, song sparrow	Boone Co.	pre-1932	M and F
R	*Zonotrichia albicollis*, white-throated sparrow	Monroe Co.	12 Apr. 1886	F
R	*Zonotrichia leucophrys*, white-crowned sparrow	Boone Co.	pre-1932	M and F
R	*Junco hyemalis*, dark-eyed junco	Boone Co.	pre-1932	M
71.8.441	*Calcarius pictus*, Smith's longspur	Benton Co.	pre-1932	M
71.8.442	*Calcarius lapponicus*, Lapland longspur	(Boone Co.)	(pre-1898)	(6)
71.8.443	*Plectrophenax nivialis*, snow bunting	St. Joseph Co.	pre-1932	F
71.8.444	*Plectrophenax nivialis*, snow bunting	St. Joseph Co.	pre-1932	M
D and R	*Dolichonyx oryzivorus*, bobolink	Boone Co.	pre-1932	M (D) and F (R)

TABLE 1.—Continued

Cat #/Rec.	Name	Locality	Date	Comments
R	*Agelaius pheoniceus*, red-winged blackbird	Boone Co.	pre-1932	M and F
R	*Sturnella magna*, eastern meadowlark	Boone Co.	pre-1932	M and F
71.8.398	*Xanthocephalus xanthocephalus*, yellow-headed blackbird	Porter Co.	pre-1898	M (6)
71.8.407	*Quiscalus quiscula*, common grackle	Boone Co.	pre-1932	F
R	*Quiscalus quiscula*, common grackle	Boone Co.	pre-1932	M
R	*Molothrus ater*, brown-headed cowbird	Boone Co.	pre-1932	M and F
R	*Icterus spurius*, orchard oriole	Boone Co.	pre-1932	M
R	*Icterus spurius*, orchard oriole	Vigo Co.	25 May 1889	M (imm.) and F
R	*Icterus galbula*, northern oriole	Boone Co.	pre-1932	M and F
71.8.403	*Icterus galbula*, northern oriole	?Boone Co.	pre-1932	F; ?specimen listed above
71.8.446	*Carpodacus purpureus*, purple finch	Vigo Co.	2 Apr. 1889	M
71.8.433	*Loxia curvirostra*, red crossbill	Boone Co.	pre-1932	F
R	*Loxia curvirostra*, red crossbill	Boone Co.	pre-1932	M
71.8.430	*Carduelis flammea*, common redpoll	Jasper Co.	pre-1932	M (6)
R	*Carduelis pinus*, pine siskin	Monroe Co.	29 Dec. 1886	M
R	*Carduelis tristis*, American goldfinch	Boone Co.	pre-1932	M and F
71.8.390	*Passer domesticus*, house sparrow	Boone Co.	pre-1932	F
R	*Passer domesticus*, house sparrow	Boone Co.	pre-1932	M
R	*Didelphis virginiana*, Virginia opossum	Boone Co.	pre-1932	M and F
D	*Scalopus aquaticus*, eastern mole	Boone Co.	pre-1932	M
71.9.105	*Scalopus aquaticus*, eastern mole	Putman Co.; near Bainbridge	pre-1932	albino; John Michaels
D	*Scalopus aquaticus*, eastern mole	Boone Co.	9 Feb. 1898	Frank Parr, Lebanon
R	*Myotis lucifugus*, little brown myotis	Monroe Co.; Coons Cave	pre-1932	
R	*Eptesicus fuscus*, big brown bat	Marion Co.; Indianapolis	9 Mar. 1907	
R	*Lasiurus cinereus*, hoary bat	?Greene Co.	pre-1932	W.B. Vangorder, Lyons
R	*Lasiurus cinereus*, hoary bat	Lake Co.	pre-1932	
R	*Sciurus carolinensis*, gray squirrel	Boone Co.; Lebanon	pre-1932	M
71.9.115	*Sciurus carolinensis*, gray squirrel	unlisted; or Brown Co., near Morgantown	pre-1932; or 27 Sep. 1930	albino; no data, or W.J. Kesterson, Indpls.

R	*Sciurus niger*, fox squirrel	Boone Co.	pre-1932	F
R	*Tamiasciurus hudsonicus*, red squirrel	Boone Co.	pre-1932	M and F
R	*Spermophilus tridecemlineatus*, thirteen-lined ground squirrel	Benton Co.	pre-1932	M and F
R	*Spermophilus franklinii*, Franklin's ground squirrel	Benton Co.	pre-1932	M and F
R	*Tamias striatus*, eastern chipmunk	Boone Co.	7 Apr. 1897	M
R	*Tamias striatus*, eastern chipmunk	Boone Co.	pre-1932	
R	*Glaucomys volans*, southern flying squirrel	Boone Co.	pre-1932	M and F
R	*Microtus pinetorum*, woodland vole	Marion Co.; Crow's Nest	9 May 1897	
D	*Microtus pennsylvanicus*, meadow vole	Boone Co.	pre-1932	two M, one F
R	*Rattus norvegicus*, Norway rat (once identified as *Rattus rattus*, black rat)	Floyd Co.; New Albany	1904	J.H. Lemon, New Albany (9)
71.9.122	*Rattus norvegicus*, Norway rat	Boone Co.	Mar. 1931	
D	*Rattus norvegicus*, Norway rat	Boone Co.	pre-1932	F
71.9.80, and R	*Rattus norvegicus*, Norway rat (once identified as *Nyctomys decoloratus*, red rat)	Boone Co.; or Marion Co., Indianapolis	pre-1932; or 1 Apr. 1903	is one of two specimens: Boone (F), Marion (M) (9)
71.9.120	*Ondatra zibethicus*, muskrat	Marion Co.	pre-1932	M; albino
71.9.123	*Erethizon dorsatum*, porcupine	(Porter Co.)	(pre-1898)	M (3,9,20)
R	*Erethizon dorsatum*, porcupine	(Porter Co.)	(pre-1898)	F (3,9,20)
71.9.128, and R	*Canis* cf. *C. lupus* × *C. latrans*, gray wolf/coyote hybrid (?listed as gray wolf, *Canis occidentalis*)	?LaPorte Co.	?pre-1909	M and F; Hon. S. Durrow, LaPorte (9)
R	"wolf"	Hamilton Co.	Apr. 1920	
R	*Vulpes vulpes*, red fox	Marion Co.; Riverside Park Fish and Game Hatcheries	26 Mar. 1919	M
71.9.129	*Vulpes vulpes*, red fox	?Morgan Co.	pre-1932	Jas Moore, Waverly
71.9.132	*Procyon lotor*, racoon	Jefferson Co.; near Madison	pre-1932	M; albino
71.9.138	*Mustela frenata*, long-tailed weasel	Boone Co.	pre-1932	M
71.9.139	*Mustela frenata*, long-tailed weasel	Boone Co.; Advance	pre-1932	F
71.9.143	*Mustela vison*, mink	Boone Co.	pre-1932	
71.9.150	*Lutra canadensis*, river otter	(Pike Co.)	(1904)	(9)

The original listing for turtles consisted of 155 entries, with 125 specimens of Indiana occurring taxa. These provided for 117 Indiana/Illinois records and 80 documented specimens. Fourteen specimens are "stuffed;" the remaining are dried shells. Most of the records for Grand Chain in the Wabash River, Posey County, IN, and for Mount Carmel, Wabash County, Illinois, appear to be from the collecting effort of W.S. Blatchley in 1901-1902 (18, p. 188), then State Geologist in charge of the Museum. Many shells examined did contain crumpled paper with original data. One of the earliest items in the Museum is a rodent-gnawed skull of a large alligator snapping turtle (71.983.115), "said to be from Arkansas," donated in March 1877, and cited by Hay (10). The mounted specimen of eastern mud turtle (*Kinosternon subrubrum*) is among the few specimens from northwestern Indiana and the only confirmed record from Starke County. The record of ornate box turtle (*Terrapene ornata*) Posey County, is noteworthy in that there is only one other record (Daviess County) for this prairie species in southwestern Indiana (18). The 1932 catalogue clearly distinguishes between a "terrapine" (*T. ornata*) and the "common box turtle" (*T. carolina*). Given the sparsity of map turtle (*Graptemys geographica*) and sawback turtle (*G. pseudogeographica*) records for southwestern Indiana and the sympatric occurrence of two subspecies (or species) of the latter in Indiana (19), specimens from these earlier populations appear important. There is a series of red-ear turtle (*Pseudemys scripta elegans*) carapaces, displaying several stages of development and variation in markings. The catalogue lists three specimens of "*Pseudemys troosti* (Holbrook)," as understood by Hay (10), from Grand Chain, Posey County, that can be attributed to three melanistic *P. s.elegans* specimens (two "stuffed," 71.7.3.29-.30; one shell, 71.7.3.11). Melanism is more frequent in older male red-ears (7; 18). One specimen from Grand Chain (71.7.1.34) displays a hunch-back condition (kyphosis) and is the largest red-ear that Minton had ever seen (Minton, pers. comm., 28 March 1985). The record of a red-ear turtle from Lake Maxinkuckee, Marshall County, is among the few from north central Indiana. Minton (18) also listed an unconfirmed Marshall County record. The limited number of Indiana hieroglyphic turtles (*Pseudemys concinna hieroglyphica x. P. floridana hoyi*) in collections increases the value of the Grand Chain/Mount Carmel specimens at the Museum. Once thought to be extinct in the state (18), there are restricted populations of this turtle in extreme southwestern Indiana (19). Thirteen specimens of turtles are "stuffed" (snapping turtle; mud turtle, Starke County; 2 Blanding's turtles,? Starke County; 2 map turtles; sawback turtle; 3 red-ear turtles (2 melanistic, Grand Chain); 3 hieroglyphic turtles), the last 9 emydids probably from Grand Chain or Mount Carmel.

The mounted bird collection contained 462 specimens representing 301 Indiana records; 90 documented Indiana specimens survive, as well as numerous probable Indiana specimens that have been separated from their data. The American white pelican (*Pelecanus erythrorhynchos*) specimen provides an early spring migration date (20 April 1896, Fountain County; 11). This specimen was previously noted (4). The brown pelican (*Pelecanus occidentalis*; Figure 1A) specimen was documented by Blatchley (4), seen by Mumford in the State Museum at the State House in 1958 (21) and presumed lost (11). This specimen is among the few accidental records of this species from Indiana. Specimens of two Indiana double-crested comorants (*Phalacrocorax auritus*) are noteworthy, as this once regular migrant is now a rare migrant, though casual in summer and winter. Butler (6) refers to two Boone County least bitterns (*Isobrychus exilis*) in the old State Museum collection; one, dismounted (71.8.34) is present. Butler (6) also refers to a whistling swan (= tundra swan, *Cygnus columbianus*) from Decatur County in the office of the State Geologist. There is only one Museum record for

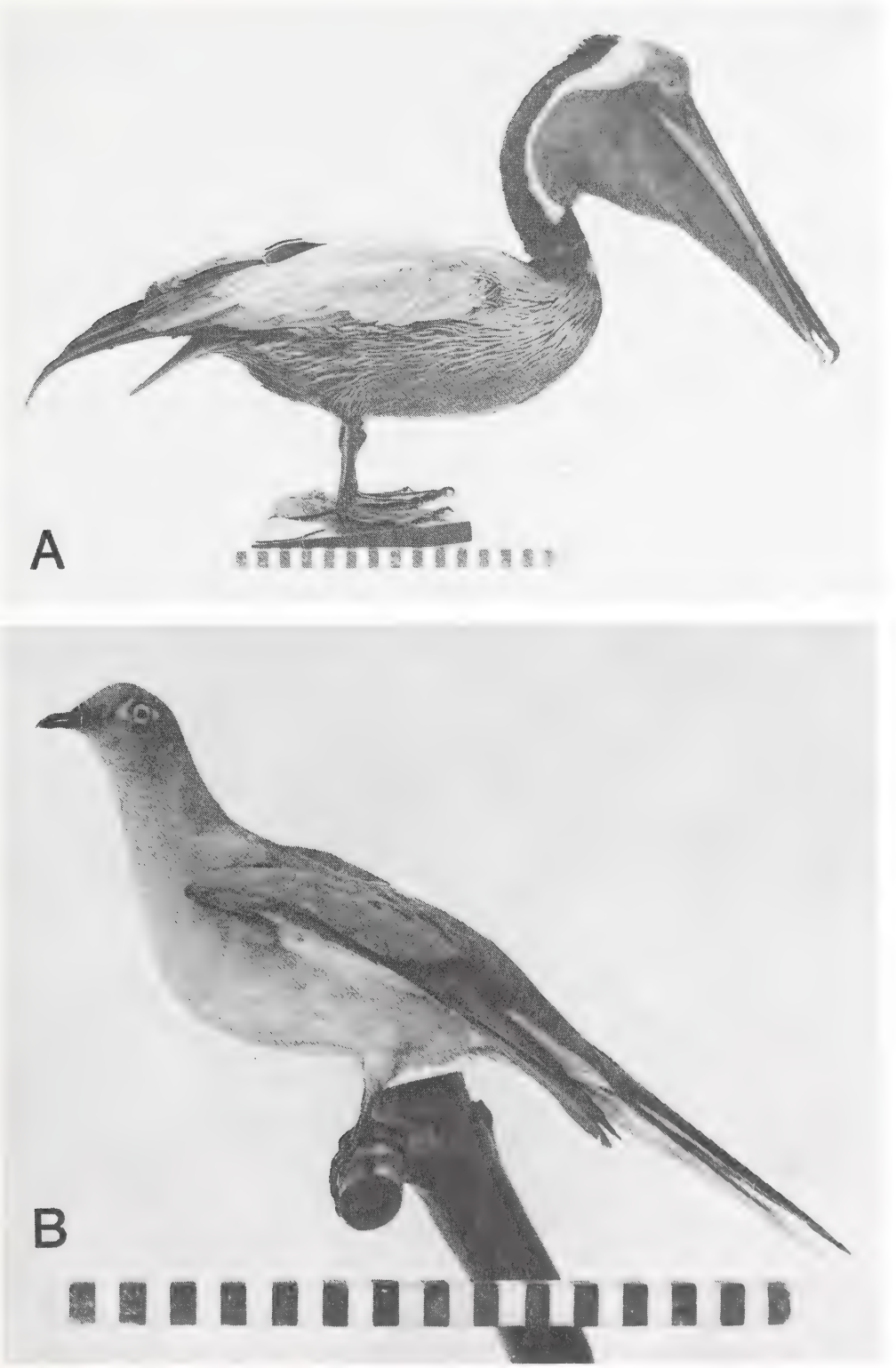

FIGURE 1. A. Brown pelican (*Pelecanus occidentalis*), collected near Broad Ripple, Marion Co., IN, 26 March 1907 (71.8.25).

B. Passenger pigeon (*Ectopistes migratorious*) collected in Wayne Co., IN, 1869 (71.8.286).

this bird (Newton County, near Moracco, killed 14 October 1897) suggesting that the more complete Newton County data is valid. The snow goose (*Chen caerulescens*) in the collection has a barring pattern, that suggests mixing with a domestic goose, perhaps even pen rearing. Snow Geese are not uncommon migrants through Indiana. The old-squaw (*Clangula hyemalis*) specimen, Boone County, represents an uncommon report for central Indiana. Indiana ospreys (*Pandion haliaetus*) were rarely available for mounting. John Michaels (a collector friend of W.S. Blatchley; 5) took the specimen (with limited data) in his home county (Putnam). Michaels also took a bald eagle (*Haliaeetus leucocephalus*), from Putnam County. Mounted bald eagle specimens from Indiana are uncommon. The old catalogue distinguishes two types of rough-legged hawks ("Black Hawk, rough-legged hawk, *Archibuteo ferrugineus*," and "Rusty hawk, Black Hawk, *Archibuteo Sanctijohannis*"). The confusing entry suggests that the ferruginous hawk (*Buteo borealis*), of rare occurrence in Indiana, may be represented by the Brown County listing, since the Boone County female appears to be the black phase of rough-legged hawk (*Buteo lagopus*). The golden eagle (*Aquila chrysaetos*) specimen from Shelby County appears to be a soundly documented, rare Indiana mount. The specimen of the uncommon merlin (*Falco columbarius*), Boone County, had been noted in the old collection by Butler (6).

The mounted peregrine falcon (*Falco peregrinus*), lacking data in the catalogue, appears to be the Boone County (Slabtown, 14 May 1896) specimen noted in the collection by Butler (6). Because the greater prairie-chicken (*Tympanuchus cupido*) is now extirpated in Indiana (last seen 1970, 21), museum specimens of Indiana stock are important. Although Butler (6) noted the Benton County specimen in the collection, the Fountain County bird at the INSM has not previously been recognized. The two Missouri specimens of wild turkey (*Meleagris gallopavo*) are listed because they represent wild stock, unmixed with more recent domestic varieties (24). A number of "experimentally" introduced birds were apparently raised on the State Game Reserve farm at Waveland, including the silver pheasant (*Gennaeus nycthemerus*), golden pheasant (*Chrysolophys pictus*) and the Texas bobwhite (*Colinus virginianus texanus*), the last taxon only represented in the existing collection. Specimens of the king rail (*R. elegans*) and Virginia rail (*Rallus limicola*) are noteworthy, given the sparsity of their records in central Indiana. The sandhill crane (*Grus canadensis*) with record only, was taken in Montgomery County during a rather late spring migration (16 April 1900). Butler (6) noted a semipalmated plover (*Charadrius semipalmatus*) from Jasper County in the old Museum collection; it is not present in the collection today. The marbled godwit mount (*Limosa fedoa*) represents a species that was once abundant in the Kankakee River marshes of northern Indiana (21) but now is a rare to casual migrant. Although the Wilson's phalarope (*Phalaropus tricolor*) specimen lacked any catalogue data, Butler (6) noted that the old INSM specimens were collected near Lebanon in Boone County, 9 May 1889.

Two red-necked phalaropes (*Phalaropus lobatus*) in the old collection were noted by Butler (6) as having been taken in Boone County, (7 June 1889); the Museum catalogue lists only two specimens from Brown County. Inclusion of a date suggests that the Boone County locality is more probable for these rare migrants in Indiana. The red phalarope (*Phalaropus fulicaria*) record (without specimen) for Daviess County is rather far south for this casual fall migrant of the Lake Michigan region in Indiana. The herring gull (*Larus argentatus*) specimen was collected from Marion County (February 1899), at a time when the bird was a winter visitor or resident (21). An extensive account was given by Butler (6) of the occurrence and collection of the thick-billed murre (*Uria lomvia*; Figure 2) in Marion County, (13 December 1896). Mumford and Keller (21) refer to a specimen taken near Pickard (Clinton County) as being "for some time" in the State Geologist's office, Indainapolis, but none

refer to the existence of the Marion County specimen. The catalogue clearly notes Marion County, 13 December 1896 for this rare mount of an accidental visitor to Indiana. Given the exhaustive scrutiny for records of the extinct passenger pigeon (*Ectopistes migratorius*) in Indiana (6; 21), it seems ironic that the INSM mounted specimen from Wayne County, collected 1869 (Figure 1B) donated by Hon. Walter

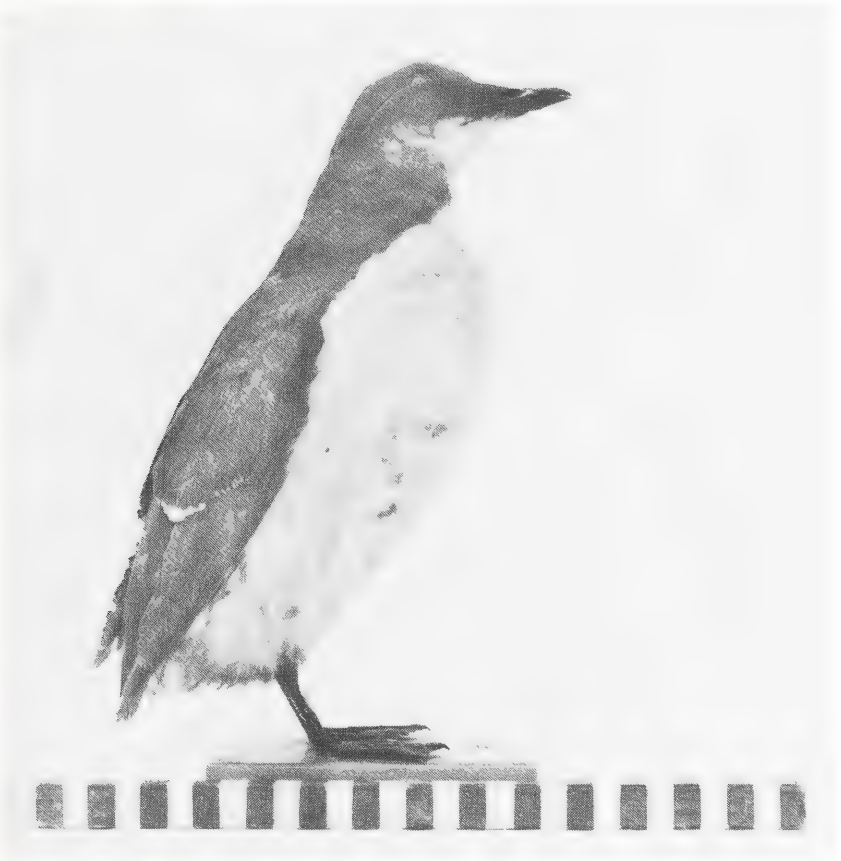

FIGURE 2. Thick-billed murre (*Uria lomvia*), collected in Marion Co., IN, 13 December 1896 (71.8.279).

S. Ratliff, Richmond, IN, 1921 (13) has been overlooked. It is in good condition. Butler (6) records a specimen of barn owl (*Tyto alba*) in the old collection from Franklin (Johnson County) taken December, 1883; by elimination, 71.8.294 is either that specimen, or another from Boone County. This specimen and two others from central Indiana become more important museum specimens as Indiana barn owl populations decline. The black-backed woodpecker (*Picoides arcticus*) was first collected by H.L. Stoddard in Lake and Porter counties in March, 1917, and four other winters through October, 1927 (21). Although not present in the collection, the catalogue shows a specimen collected at Mineral Springs, Porter County, (21 November 1920), donated by Stoddard himself. The record of Bohemian waxwing (*Bombycilla garrulus*), collected at Miller's Lake (?Porter County) by H.L. Stoddard is interesting, since the bird is a rare and irregular winter visitor to Indiana. Mumford and Keller (21) refer to a northern shrike (*Laninus excubitor*), in the old collection. The Museum catalogue confirms its data (Brown County, 11 October 1893), but the specimen is

missing. There is also a Museum record for this rare central Indiana winter resident for Monroe County. Catalogue records exist for a pair of loggerhead shrikes (*Lanius ludovicianus*) for Boone County and there is a specimen in the collection without data, but it is probably the loggerhead shrike listed in the undocumented collection of Dr. Eugene Buehler, donated 16 April 1932, since the catalogue number does not match the Boone County records. The black-throated blue warbler (*Dendroica caerulescens*) specimen, Boone County, is an interesting record for this uncommon migrant. The pine warbler (*Dendroica pinus*) from Warren County was in poor condition and dismounted for storage; this bird is presently a rare migrant and very rare summer resident in northern northern (11). Butler (6) refers to two prairie warblers (*Dendroica discolor*, Boone and Starke counties) in the old collection; the Museum catalogue lists only two Boone County entries, and there are no specimens. The prairie warbler is a rare migrant and locally uncommon summer resident in central Indiana (11). Butler (6) refers to a Boone County specimen of Wilson's warbler (*Wilsonia pusilla*) in the old collection; the head is missing and it has since been dismounted for storage. The bird is presently an uncommon spring and common fall migrant. The lark sparrow (*Chondestes grammacus*) specimen from Monroe County is interesting, since Butler considered it rare at the time when it was probably collected; it is presently an uncommon to rare summer resident in southern Indiana. Butler (6) noted a savannah sparrow (*Passerculus sandwichensis*), Starke County, in the old collection; we have the record at the Museum, but not the specimen. It is a common Indiana migrant. Butler (6) lists a specimen of LeConte's sparrow (*Ammodramus leconteii*), Boone County, in the old collection; there is a record only of this rare Indiana migrant. An undocumented Lapland longspur (*Calcarius lapponicus*) mount may be one of the Boone County pair noted by Butler (6). It is an irregular and uncommon migrant in Indiana. The specimen of Smith's longspur (*Calcarius pictus*), Benton County, is noteworthy, as it is presently an uncommon spring migrant in northwestern Indiana. The yellow-headed blackbird (*Xanthocephalus xanthocephalus*) specimen, Porter County, is one of a pair listed by Butler (6). The bird was a local summer resident around the turn of the century or early 1900's and is now a rare and local resident in Lake and Newton counties. The common redpoll (*Carduelis flammea*) specimen from Jasper County was cited by Butler (6); it is presently an irregular winter visitor.

A number of birds with potentially interesting Indiana occurrence remain undocumented in the 1932 catalogue. They are listed, should data emerge: red-throated loon (*Gavia stellata*), 71.8.13) red-necked grebe (*Podiceps grisegena*) 71.8.14; American swallow-tailed kite (*Elanoides forficatus*) 71.8.140; two bald eagles (*Haliaeetus leucocephalus*) 71.8.161 and 71.8.165; golden eagle (*Aguila chrysaetos*) 71.8.155, coll. 16 Jan 1904 by Jefferson Bugg; several gray partridges (*Perdix perdix*) 71.8.176-.180; two greater prairie chickens (*Tympanuchus cupido*) 71.8.210 female, 71.8.212 male; purple gallinule (*Porphyrula martinica*) 71.8.237; black-billed magpie (*Pica pica*) 71.8.344; common raven (*Corvus corax*) 71.8.346; yellow-throated vireo (*Vireo flavifrons*) 71.8.371; Lincoln's sparrow (*Melospiza lincolnii*) 71.8.445; yellow-headed blackbird (*Xanthocephalus xanthocepholus*) 71.8.397; pine grosbeak (*Pinicola enucleator*) 71.8.445. It is interesting that a sora (*Porzana carolina*) 71.8.235 displays partial albinism, the full condition shown by two crows (*Corvus brachyrhynchos*) 71.8.347 and 71.8.644.

The mammal catalogue consists of 95 non-skeletal listings. (Several undocumented and sometimes exotic skulls were of only general interest.) Seventy-three specimens were of Indiana-occurring species; of the 39 documented Indiana specimens, only eleven remain. The mounted specimen of "black rat" (*Rattus rattus*) collected at New Albany (Floyd County) in 1904 (9), has subsequently been reidentified as a Norway

rat (*R. norvegicus*) (20). The whereabouts of the specimen is unknown. Two records of the "red rat" (*Nyctomys decoloratus*) from Boone and Marion counties, cited by Hahn (9) in the old collection, are represented today by one skull (71.9.80). It was removed from a deteriorated mount labeled "red rat", locality unspecified. The skull appears to be that of a Norway rat (*Rattus norvegicus*). Two porcupines (*Erethizon dorsatum*), listed without data, are represented by one specimen. Blatchley (3) referred to two LaPorte County specimens in the State Museum. They are mentioned by Hahn (9), and Mumford (20) noted that only one specimen remained. This may be the only extant non-skeletal specimen of Indiana porcupine. The catalogue lists three wolves, two from LaPorte County and one from Hamilton County; there is only one undocumented mounted "wolf" present in the collection. It appears too large for the coyote (*Canis latrans*), too small for a timber wolf (*Canis lupus*), yet has a pelage not unlike either (Christine Stewart-Marks, pers. comm., April 1987). Hon S. Durrow of LaPorte, who hunted wolves and coyotes, believed that some specimens from northern Indiana were coyote-wolf hybrids (9). He further related that many of the "wolf" reports that he investigated in northern Indiana actually referred to the coyote. It is possible that the Museum specimen represents a coyote-wolf hybrid, and one of the "wolves" donated from LaPorte County by Durrow himself. Lyon (17) believed that 1908 was the latest date for the timber wolf in Indiana; if correct, then the record of Hamilton County "wolf", collected 1920, was probably a coyote. A mounted river otter (*Lutra canadensis*), with a badly distended skin, has no catalogue data. Hahn (9) related that it was a Pike County Specimen, taken in 1904. It may be the only extent non-skeletal specimen of Indiana river otter. A bobcat (*Felis rufus*) mount (71.9.151) is without data, but is noted, should data emerge. Several albino mammals are present: eastern mole (*Scalopus aquaticus*) 71.9.105; muskrat (*Ondatra zibethicus*) 71.9.120; gray squirrel (*Sciurus carolinensis*) 71.9.115; and raccoon (*Procyon lotor*) 71.9.132. One opossum (71.9.103) is of light color, one raccoon (71.9.133) is "red", and a mink (*Mustela vison*, 71.9.142) has light underfur ("cotton mink"). A striped skunk (*Mephitis mephitis*, 71.9.148) has a darkened, poorly developed stripe. Mumford and Whitaker (22) comment on pelage variation in these mammals.

Discussion

The early State Geologists such as Cox, Collett and Thompson (12), who were in charge of the State Museum ("cabinet") greatly expanded the invertebrate fossil, mineral and archaeological collections through survey work. Gorby (8) noted additions to the natural history department by taxidermist J.E. Beasley of Lebanon, Boone County. Though undated records in the 1932 museum catalogue are referred to as "pre-1932", it was during the tenure of Willis S. Blatchley, biologist appointed State Geologist (1894-1911), that the bulk of the vertebrate collections were amassed. Blatchley (1) felt that the Museum should contain "examples of the different objects of Natural History found within the State", and noted that many birds and mammals were rapidly becoming extinct and should be procured while they were available. He related (2) that over 200 mounted birds and mammals and a large collection of bird's eggs had been acquired in the past two years. Most of this mounting was the contracted work of J.E. Beasley of Lebanon, resulting in a preponderance of local, Boone County, specimens. The apparent strategy was to acquire mounts of both sexes of each bird and mammalian species. Fish from his survey of Walnut Creek and tributaries are noted to have been placed in the State Museum (5), but those specimens are not now in the collection. Blatchley accumulated an extensive vertebrate collection, well-furnished in mounted bird and medium-sized mammals, bird eggs, "stuffed" turtles and their dried shells, and a small collection of liquid preserved fishes and herptiles.

Little attention was given to study skins of birds or mammals. It was in those Blatchley years that Butler (6) and Hahn (9) examined birds and mammals in the rapidly growing collection. Subsequent return of control of the State Museum to geologistss understandably put a damper on the expansion of systematic biological collections. In the 1920's and 1930's the museum collections were so shuffled from room to room, resulting in lost and damaged specimens and records (25), that the 1936 Lyon found the mammal collection scientifically useless (17). With much to the survey work completed, as amassed in the numerous volumes of the Department of Geology and Natural Resources, the Department was reorganized into the Department of Conservation, and the museum was moved into the basement of the State House (12). Unlike previous decades the Museum apparently undertook little field collection of natural history specimens. Through public donation cultural history artifacts began to accumulate in volume (e.g., 14, 15, 16). The menagerie of historical items, often with poor documentation, continued to accrue in grossly insufficient facilities, and began to resemble a great chamber of oddities and curiosities. The constant struggle for adequate storage, security and record control continued until the collections were relocated in its present facility in 1966. Here, the general collections began to receive more effective management. Mounted and freeze-dried vertebrate collections grew under taxidermists David McLary and David Tolbert, and the donations of Hayden Sharrett and Leonard Boehning. New and old vertebrate collections were curated primarily for their display value until about 1981 when the scientific value of the natural history collections began to be assessed (26). Like early collections at many institutions, specimens frequently have little, if any, documentation, and are often deteriorated. Interestingly, 28% of the mammals, 29% of the birds and 68% of the turtles with Indiana data from the "old collection" are present in the collection today. In contrast all, exceptone, of the liquid preserved fishes, amphibians and reptiles, except one have been "lost". Fortunately, the existing collection does include such important specimens as an American white pelican, brown pelican, tundra swan, two greater prairie chickens, passenger pigeon, thick-billed murre, porpucine, otter, hieroglyphic turtles and muskellunge. Valuable systematic collections require curation by staff versed in their management, conservation, data recovery and the significance of the information which the specimens provide. Mumford (20) wrote: "A new State Museum has recently been established at Indianapolis. Perhaps this institution will help increase our knowledge of Indiana mammals and other animals". The present and an earlier work (25) is the beginning of that endeavor.

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The Dollens Mastodon (*Mammut americanum*) Locality, Madison County, East Central Indiana

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Abstract

Excavation in a peat deposit, Madison County, east-central Indiana has produced 11 elements of a young adult American mastodon (*Mammut americanum*). Most of the bones were poorly preserved due to subaerial exposure after deposition; a few elements endured well enough for consolidation. Extensive washing of the surrounding sediments (approx. 8.2 cubic meters) produced only a sparse semiaquatic fauna.

A pollen profile which included pollen of both submersed and emergent aquatic plants and remains of algae indicates that the bones were deposited in a grass-sedge fen which maintained standing water for much of the year. Activities of beaver (*Castor canadensis*) and muskrat (*Ondatra zibethicus*) were apparent. The predominant arboreal pollen type is spruce (*Picea*), with some hardwood genera present. Wood from a peat layer immediately below the mastodon skull gave an age of $12{,}240 \pm 80$ ybp. More extensive analysis of pollen and plant macrofossils is in progress.

Introduction

On August 3, 1986 crane operator Mike Jordan uncovered some large bones while digging a recreational pond in a peat deposit 1 1/2 miles east of Alexandria (SW 1/4, NW 1/4, NE 1/4, NE 1/4, Sec. 29, T 21 N, R 8 E, Anderson North Quad.) Madison County, IN. The property owners, Randy and Cindy Dollens, contacted the Archaeological Resources Management Service, Ball State University, who in turn notified the Indiana State Museum.

The bone deposit occurred in a peat accumulation on the Tipton Till Plain, a flat to gently rolling plain (23), locally formed on tills of the Trafalgar Formation (31; Fig. 1A). The Trafalgar tills were depostied during the fourth advance of the Huron-Erie Lobe of the Laurentide ice sheet (Woodfordian Phase II, after 21,000 ybp; 6). The site occurs less than 1 km southwest of the Union City Moraine, a recessional feature of Woodfordian Phase III, deposited by 14,000 ybp. The sediments of the Dollens locality are typical of those of the paludal facies of the Martinsville Formation, usually deposited within the past 13,000 years (30). The bone bearing peat represents terminal sedimentation of a "kettle" lake basin, formed by the melting of accumulated ice within the Wisconsinan till (24). These peat and often associated marl deposits favored the accumulation of Quaternary vertebrates. Remains of the

FIGURE 1. A. View (to west) of mastodon (*Mammut americanum*) bone occurrence, Dollens locality, Madison County, IN. Note plaster mold enclosing tusk. Pond previously excavated in peat is in upper right of photo. B. Posterior surface of R femur, exposed *in stiu*. Note general deterioration of bone and fragmentation of proximal end (to right). Scale in centimeters.

American mastodon, Jefferson's mammoth (*Mammuthus jeffersonii*) and giant beaver (*Castoroides ohioensis*) have previously been reported for Madison County (9; 16; 20).

Because much of the Dollens skeleton appeared undisturbed, it was thought that associations of other fauna, pollen and plant macrofossils might provide information on late Pleistocene paleoecology of the region. An excavation jointly sponsored by Ball State University and the Indiana State Museum was undertaken from August 6 through 13, 1986. Field labor was supplied by staff and volunteers of both institutions, as well as by volunteers from the site vicinity. Materials have been deposited in the Indiana State Museum, catalogue numbers 71.3.39 (mastodon) and 71.3.40 (other fauna/flora).

Methods

Large elements disturbed by the crane (mandible, L tusk, L scapula blade, L humerus, proximal end) were immersed in a water tank to prevent drying and cracking. To expedite entry into and below the bone levels, undisturbed overburden was stripped by heavy machinery to within 0.5 m of the top of the bone bed. A grid of eight two meter squares was staked out with transit and tape and a site map prepared. Large bones were located with steel probes and marked with pin flags. Entry into the bone level was by shovel, where bones were exposed with trowels and bamboo picks (Fig. 1B). Small bones were mapped and removed; large bones were left in place until all located elements were exposed (Fig. 2). Bones were covered with plastic bags to prevent drying, but required periodic moistening. Stratigraphy and slope was difficult to recognize in the poorly differientiated peat. Only one vertical datum, the interface of course brown peat overlying a more fibrous, greenish (while unoxidized) peat, occurred no less than 20 cm below the bone level, and sloped southwesterly into the apparent peat basin. The level containing the mastodon bone was considered as a single depositional unit. Elevation of individual bones was recorded, though not in relation to the peat interface. A 20 cm balk was left in the corner of each unit for pollen and microfauna samples. Pollen samples were removed at 5 cm intervals down one balk, spanning the R tusk tip, as well as from the tusk cavity. A core of 3.6 m of lake sediment beneath the peat layer was taken at a later date. Select wood samples were sealed in aluminum foil for radiocarbon dating. The fragile R tusk was molded *in situ* in plaster reinforced with burlap; this later provided a fiberglass cast of the tusk. After the exposed elements were photographed, mapped and numbered, they were sealed in plastic bags for transport. After all bones were removed, Unit 3 was deepened by 38 cm (encountering greenish peat at 10 cm), and a 54 cm^2 pit dug an additional 81 cm; coring 91 cm deeper revealed greenish peat and no bone. A final collapsing and backfilling of all unit floors 50 cm deeper with shovels revealed no large bone.

Sediment removed from the units was transported by wheelbarrow to the washing station ca. 100 meters away. A total of 8.1 cubic meters was washed through 6 mm mesh hardware cloth for recovery of plant and animal macrofossils. Water was pumped from an inundated dragline trench by a gasoline engine pump with a 3.81 cm (1 1/2″) delivery hose.

In the Museum, bones were lightly brushed under tap water, then sealed in plastic bags for slow drying (2-3 months). Dried bones and teeth were later immersed 2-3 times in a ca. 15% solution of glyptal, carried by acetone, and broken parts mended with epoxy. Most of the skull, much of the postcranial bone and some of the tusk was too disintegrated for reconstruction and cataloging into the curatorial collection. While most of the exterior laminae of the tusks did exfoliate, a fiberglass cast preserves the external detail of the R tusk. Several samples of beaver-gnawed wood were preserved by freeze-drying. Two samples of gnawed wood were air-dried to 25% of their original

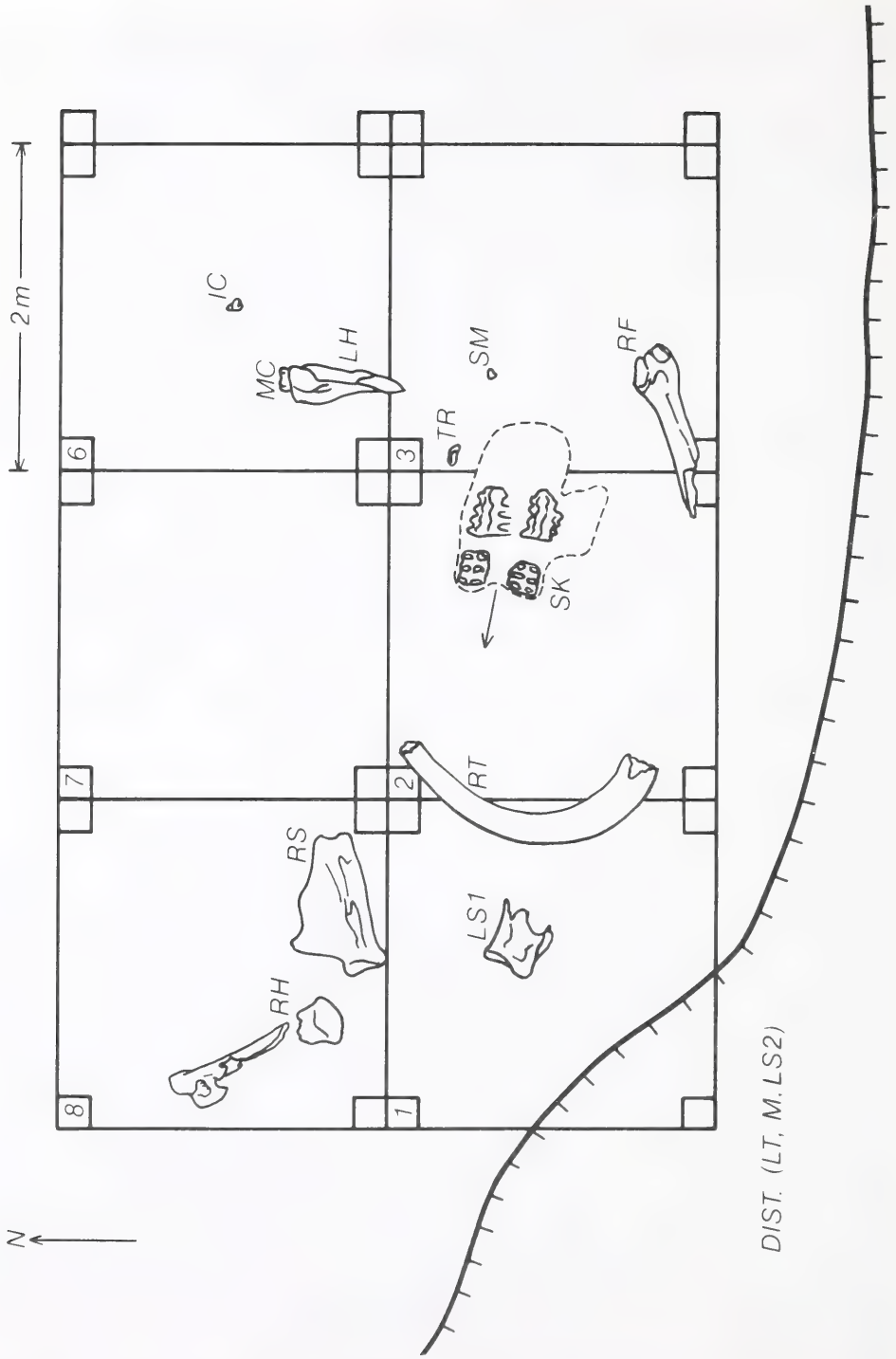

FIGURE 2. Plan view sketch of mastodon (*Mammut americanum*) remains recovered *in situ*, Dollens locality. South and southwest corner disturbed by crane upon discovery. Abbreviations: RH, right humerus; RS, right scapula; MC, right metacarpal IV (of manus); LH, left humerus; IC, left internal cuneiform (of pes); LS1, left scapula, glenoid cavity area; RT, right tusk; SK, skull, with four molars (arrow points anteriorly); TR, right trapezoid (of manus); SM, sesamoid (of manus/pes); RF, right femur; Dist., disturbed area; LT, left tusk; M, mandible; LS2, left scapula blade. Grids numbered in upper left corners.

volume. Recovered plant macrofossils were forwarded to Steve Jackson for identification. A total of .091 cubic meter (3.21 cubic feet) of peat from the balks and the greenish levels was washed through 1.2 mm mesh screen for microfauna recovery.

Results

A partial skeleton of a young adult American mastodon was recovered along with isolated elements of 7 predominantly semiaquatic vertebrate species, plant macrofossils of predominately coniferous trees, a pollen spectrum and 2 radiocarbon dates.

The American mastodon (*Mammut americanum*) elements include: skull (very fragmented) with four molars (L,R M2 and M3); two tusks (one fragmented); mandible with four molars (L,R m2 and m3) (Fig. 3); R m1; L (fragmented), R scapulae; L,R humeri (fragmented); R femur (fragmented); R trapezoid and R metacarpal IV (of manus); L internal cuneiform (of pes); sesamoid. It appears to represent a young adult skeleton. Epiphyseal union is complete distally on the humeri and femur; the proximal epiphyses of the humeri were actively closing, with fusion complete on the anterior half of the shaft. The epiphyses of the femoral head and superior border of the scapulae were unfused. The isolated Rm1, removed from a peat heap, was actively being shed, and would not have maintained itself in the remodeled alveolus without gingival support. Only the anterior roots and alveoli of the m1 remain; the posterior root had been resorbed and the alveolus closed. The shedding of the m1, and the molar wear stages (m1, A/3, M2/m2, 2+; M3/m3, 1) indicate a young adult age of 26-28 ± 2 years, using the Boney Spring mastodon age structure as a template (22). Tooth wear and eruption are roughly equivalent to stages XVII-XIX of the African elephant (28-32 years of age; 15). An age of 30 African elephant years is suggested for the Dollens mastodon.

Tooth measurements are listed in Table 1. While some mastodon population samples display small teeth (Trollinger Spring, MO, 22; Christensen Site, IN, 8), other samples (Boney Spring, MO, 22), including those of diachronous age (Michigan sample, 28) display a great range of size. The Dollens teeth are clearly of intermediate size in the range displayed by the Boney Spring and Michigan specimens. Skeletal measurements (Table II) also indicate an average-sized individual (20).

The Dollens mandible displays some traits suggestive of a female such as lack of mandibular tusks and a "U" shaped lingual gutter (22), but a sexed series of mandibles is not available for comparison.

An elliptical area (50 mm × 41 mm) of reactive bone growth is present on the midline of the L mandibular ramus, below the m3 tetartolophid. This may or may not represent a healed, puncture injury.

Many of the bones are rodent gnawed on the upper surfaces more readily exposed during burial. These include the L condyle and coronoid processes of the mandible; base of R tusk; L humerus, distal end; L scapula, anterior and lateral margins of glenoid cavity, anterior and posterior blade margins and spine border; R scapula, blade margins. The large size of the gnawings suggest such rodents as muskrat, beaver, or porcupine. The gnawing indicates subaerial weathering of the bones for some time after deposition, and with the freeze-thaw conditions of shallow burial, allowed for deterioration of the bones.

Washing produced the following sparse, predominantly semiaquatic fauna (M, indicates elements recovered from the 1.2 mm mesh):

Fish sp. (71.3.40.1). MATERIALS: Two minute, unidentified bone scraps (M).
Rana sp., frog (71.3.40.2). MATERIALS: R maxilla, anterior portion (M). COMMENTS: 50-60 mm body length.

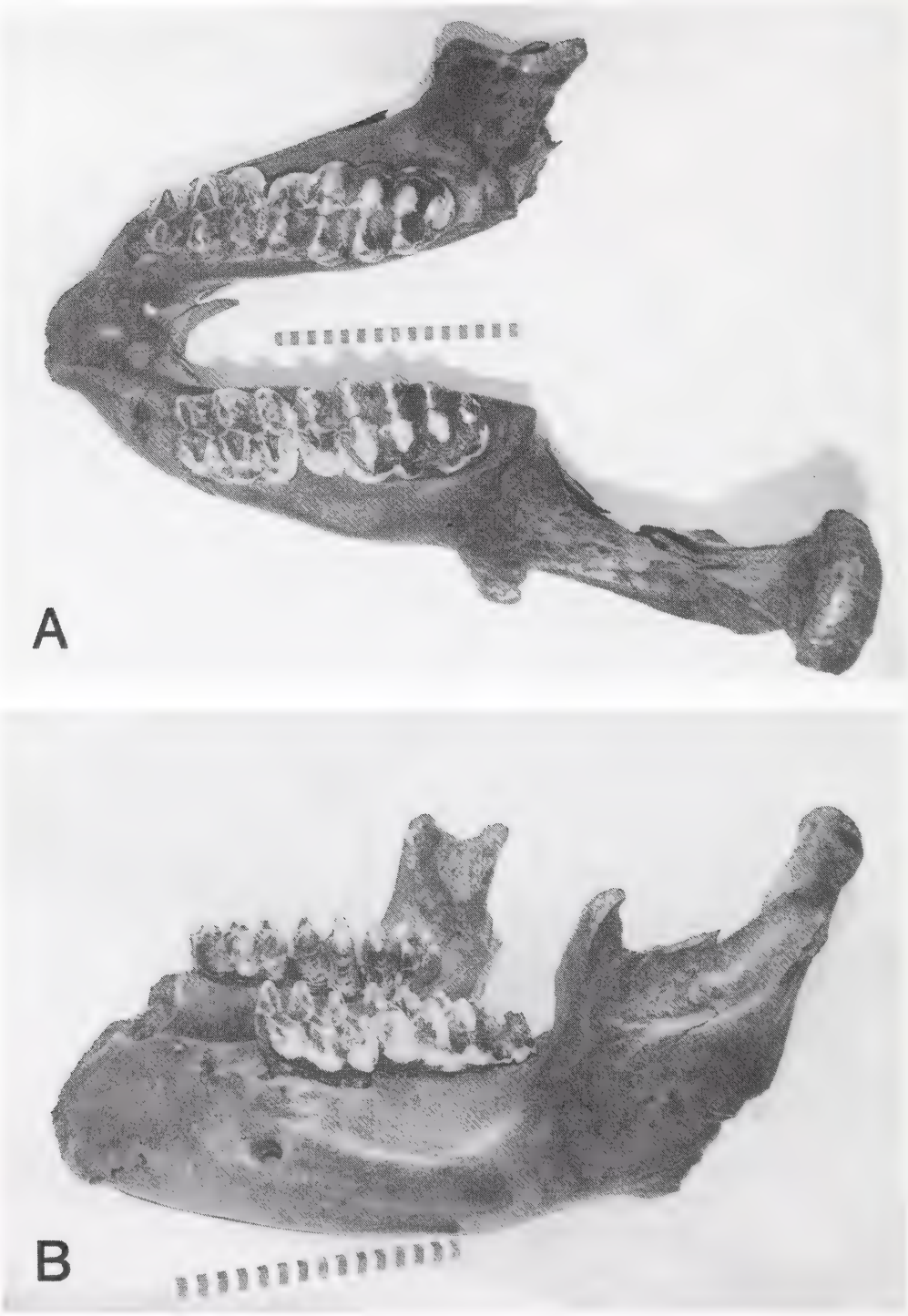

FIGURE 3. Mandible, Dollens mastodon (*Mammut americanum*). A. Occlusal view. Note alveoli for only anterior roots, indicating active shedding (or loss) of the first lower molar. Scale in centimeters. B. Left oblique view. Much of the missing coronoid areas are heavily rodent gnawed.

TABLE 1. Tooth measurements, *Mammut americanum*, Dollens Locality, (mm).[1,2]

Placement	Cat. #	Length	Width	Maximum width across:			
				Protoloph(-id)	Metaloph(-id)	Tritoloph(-id)	Tetartoloph(-id)
LM2	71.3.39.10	119.5	95.5	90.9	94.6	94.8	
RM2	71.3.39.9	121	95.0	89.4	95.0	94.1	
LM3	71.3.39.12	187.0	103.5	102.3	103.8	100.1	90.2
RM3	71.3.39.11	194.5	103.5	101.2	106.0	101.5	92.2
Rm1	71.3.39.2	98.5	74.5	62.9	76.2	71.7	
Lm2	71.3.39.1	114.5	98.0	77.7	95.8	94.5	
Rm2	71.3.39.1	112.0	94.0	77.2	94.1	93.6	
Lm3	71.3.39.1	196.0	108.0	90.4	109.1	103.0	91.9
Rm3	71.3.39.1	192.0	107.0	91.3	107.7	101.8	90.7

[1] Terminology from Saunders (22).

[2] Tusk dimensions: R tusk, preserved length (outside curve): 1920 mm; R tusk, greatest diameter (not at base): 179 mm; L tusk, 950 mm section of tip retained integrity.

Chelydra serpentina, common snapping turtle (71.3.40.3). MATERIALS: L femur, proximal end.

Castor canadensis, beaver (71.3.40.4-.6). MATERIALS: L dentary, anterior portion with p4 and m1 from the peat heap; upper molar; thoracic vertebra.

Mouse sp. Indet. (71.3.40.20). MATERIALS: upper incisor enamel fragment (M).

Ondatra zibethicus, muskrat (71.3.7-.19). MATERIALS: R dentary with i; Lm1; LM1; R nasal, posterior portion (M); 10 cheek tooth enamel plates (M); caudal vertebra; R humerus; L innominate, fragmented; L femur, proximal end; R, fragmented L tibiofibula. COMMENTS: Tibiofibulae from different individuals. Enamel plates represent disintegrated cheek teeth.

Sciuridae cf. *Sciurus* sp., tree squirrel (71.3.40.21). MATERIALS: distal phalange (M). COMMENTS: Recovered from the upper deciduous pollen zone.

TABLE II. Skeletal Measurements, *Mammut americanum*, Dollens Locality (mm)

Element/Measurement	Left	Median	Right
Mandible			
Height of condyles above lower border of jaw:	466		
Length of symphysis:		146+	
Height of jaw at front of m3:	169		177
Thickness of jaw at front of m3:	150		147
Greatest thickness of jaws (through anterior insertion of ascending ramus):	173		ca. 168
Scapula			
Greatest length from coracoid process to top of scapula, along axis of spine (lacking epiphysis):			806
External distance from inner border of glenoid cavity to top of spine (lacking epiphysis):			770
Greatest anteroposterior length of glenoid cavity:	217		219
Greatest transverse width across glenoid cavity:	138		143
Humerus			
Distal end, transverse width:			289
Distal end, anteroposterior depth (of medial condyle):			207
Femur			
Least transverse diameter of shaft:			169
Anteroposterior diameter of shaft at above position:			83
Greatest transverse width of distal end (normal to articular surface):			260
Least circumference of shaft:			413

The recovered fauna indicates a marshy wetland with emergent vegetation and probably nearby permanent water at the time of mastodon deposition. Aquatic vertebrates such as fish and salamanders were apparently sparse in the marsh, due perhaps to periodic drying. There was little evidence for turtles, and none for snakes, mink, or even the meadow vole (*Microtus pennsylvanicus*), usually common in marshes, meadows and around lake shores (18).

The lower, greenish peat level was C-14 dated at 12,650 ± 120 ybp (Beta-17594), and wood from immediately below the skull at 12,240 ± 80 ybp (Beta-20143). The possibility of sediment disturbance by the living mastodon (7), and tendency of bones to settle downward through the peat (5), suggest that the 12,240 ybp date is a maximum for mastodon deposition.

Some pieces of burned wood were recovered in Units 2 and 8 spanned by, though not necessarily contemporaneous with, the mastodon bones.

A set of 9 pollen samples spanning 95 cm of an exposed peat face in which one tusk was embedded has been counted. The pollen spectra indicate that the bones were depostied in a marshy peat-forming fen in which there was standing water for much of the year. This setting is suggested by high percentages of grass and sedge, the occurrence of many aquatic macrophytes (*Myriophyllum, Typha latifolia, Brasenia, Sagittaria, Utricularia, Nuphar*, and *Potamogeton*) and remains of several algae (*Botryococcus, Pediastrum boryanum, P. araneosum, P. integrum*).

The tree pollen types are clearly dominated by spruce with some larch and fir. These taxa are indicative of boreal conditions. Some hardwoods also occurred. The most common hardwoods were ash, ironwood-hornbeam, elm, and oak. Pollen spectra similar to these are found from a number of late-glacial sites on the till plains of Indiana and adjacent Ohio (1; 25; 32; 33). At most sites the late glacial transition from spruce dominance to spruce and some hardwoods occurs between 13,500 and 12,000 ybp. This is consistent with the available radiocarbon dates from the Dollens site.

At many midwestern mastodon localities the forests were apparently open, spruce-dominated boreal woodlands (13; 32). This may be true for the Dollens site also, as *Ambrosia, Artemisia*, and Tubuliflorae grains occur. However, the evidence is less conclusive, as the high percentages of grass and sedge are probably from wetland taxa.

Discussion

Fauna, flora and sediments indicate the Dollens mastodon was deposited in a vegetation-choked marshy basin some 12,240 ybp or less, during the terminal infilling of a kettle lake. Seasonally dry, the area of bone deposition lacked true aquatic fauna such as fishes and mollusks, though permanent water was in the immediate area. Just prior to and perhaps during mastodon deposition the marsh was occupied by muskrat and active beaver lodges. Beaver gnawed wood, sometimes burned, and collapsed lodges have also been regularily found in the lower Late Pleistocene, levels of southeastern New England peat deposits (14).

The recovered fauna consists of current resident species, and without the presence of mastodon and conifer pollen, would not suggest an environment any different from that of the area today. Open spruce woodlands, predominant during the early late glacial in much of the Midwest (2), is the predominant environment associated with Midwestern mastodon remains (3; 13; 32).

The low faunal diversity noted in this study is also apparent in Michigan muck and peat deposits (12). Localities where mastodon bones occur in marl and sand, such as the Kolarik locality, Starke Co., IN, however, can be quite productive for mollusks, fishes and other vertebrates (20). These kettle lake and bog faunas do not

approach the abundance and diversity of taxa recovered from the more terrestrial cave deposits (19; 20; 21).

Although the right scapula and humerus were semi-articulated, the remainder of the bones, consisting mainly of larger elements, was dispersed. Major skeletal areas, such as the vertebral column, ribs, lower legs (tibiae, fibulae, ulnae, radii), pelvis and most of the foot bones were missing. Major portions of mastodon skeletons are often not found in marl (lake) and bog/marsh (peat) deposits, even where other portions are articulated (Kolarik locality, IN, Ellis and Richards, in prep.; 8; 12), perhaps because of natural processes (8). The lack of any vertebrae or ribs (over 75 elements) suggests a non-random process of bone dispersion. The Dollens bone pattern characterizes two different modes of dispersion. First, the presence of such large bones as the skull, mandible, humeri and femur (Class III and II) and lack of ribs, vertebrae and most of the podials (Class I) is typical of bones that have moved slowly (by traction) in running water (29). Except for an occasional pebble or seam of sand, there was, however, little indication of moving water in the deposit. Second, the bones present are those which, on dry land, might disarticulate early (scapulae, mandible), or later (femur, humeri, podials) from the skeleton; vertebrae, ribs and pelvis (those "missing" from the deposit) can be the last to disarticulate (10; 11). Hill (10) further suggested that units composed of more than one bone (e.g. vertebral column) do not readily scatter, and that in the absence of running water the vertebrae should be the bones remaining longest at the death site. Peat removed by crane from the deeper basin immediately to the south and west of the bones was spread by heavy machinery and failed to produce any of the missing parts. Perhaps the vertebrae, pelvis and ribs are closely associated, buried in the shallow peat to the north or east of the bones.

Fisher (4; 5) describes several Michigan mastodon sites that suggest butchery by Paleo-Indians using bone wedges to separate articulated elements. Use of the scanning electron microscope detailed differences between rodent, digger/preparator markings and those produced by proposed bone wedges and cutting instruments (26). The Dollens mastodon R humerus head does have several gouge-like marks, but lacks any matching marks on the articulating R scapula glenoid cavity. Other bones with similar marks lack conarticulating elements. We suggest that these marks were made by rodents, scavengers, and during the excavation process, including probing. The SEM was not used. Concentrate from the several cubic meters of washed sediment did produce one chert microflake with a platform (71.3.40.22, 2.8 mm × 2.0 mm × 0.8 mm) from the top 10 cm of Unit 3 (NW corner balk). Although chert microflakes would be expected at a mastodon butcher site as a result of tool resharpening or use (17), it is difficult to directly relate this microflake to the mastodon skeleton.

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Fishes of Marion County, Indiana

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The purpose of this report is to bring together available information on the fishes of Marion County, Indiana. Marion County is in central Indiana and consists primarily of Indianapolis and its suburbs. Indianapolis is one of the larger cities of the United States. It is encircled by an Interstate Highway system, I-465. Also I-70, I-65, I-69, and I-74 traverse the city in various directions.

The west fork of the White River flows across the county from NE to SW. It and its tributaries drain the entire county. Flowing into the White River from the west are Eagle Creek, Crooked Creek and Williams Creek, listed from south to north. Corresponding creeks on the east are Lick Creek, Pleasant Run and Bean Creek which come together before entering the river and Fall Creek.

A complete list of the fishes of Marion County is given in Table 1, separated by collections made in the last century, those made by Gerking in Marion County during his studies of the fishes of Indiana, and recent collections (those made since 1965). Fishes from the White River are summarized in Table 2. Fishes of Eagle Creek are listed in Table 3, Fall Creek in Table 4, several small creeks in Table 5, and fishes of the Indianapolis Water Company Canal and in Pleasant Run in Table 6.

David Starr Jordan and associates reported a number of species from the White River and its tributaries in Indianapolis in the 1870s and 1880s. These were reported in several papers, and many of the names of the fishes are outdated and hard to link with present-day species. However, Eigenmann and Beeson (3), Gerking (7), and Gammon (6) have attempted to determine correct nomenclature and have summarized data from the early literature. We have used these works to determine the species, totalling 63, recorded from Marion County by Jordan and associates during the last century. The 63 are indicated in Table 1.

The most comprehensive study of the fishes of Indiana is by Gerking (7), who made 515 collections around the state from 1940 through 1943. Gerking made 5 collections in Marion County, taking 37 species (Table 1), 9 of them new records for the county. This brought the number of species of fish known from Marion County to 72. Species taken from the first time in Marion County by Gerking were

MINNOWS - Cyprinidae

Goldfish, *Carassius auratus*
Carp, *Cyprinus carpio*
Spotfin shiner, *Notropis spilopterus*
Mimic shiner, *N. volucellus*
Suckermouth minnow, *Phenacobius mirabilis*

TABLE 1. Complete list of fishes taken from Marion County, Indiana, with indication as to whether taken in past century by Jordan and associates (1870's), by Gerking (1945), or recently (since 1965).

	1870's	Gerking 1945	Since 1965
Lampreys — PETROMYZONTIDAE			
Silver lamprey, *Ichthyomyzon unicuspis* Hubbs & Trautman	X		
Paddlefishes — POLYODONTIDAE			
Paddlefish, *Polyodon spathula* (Walbaum)	X		
Gars — LEPISOSTEIDAE			
Longnose gar, *Lepisosteus osseus* (Linnaeus)	X		
Bowfins — AMIIDAE			
Bowfin, *Amia calva* Linnaeus			X
Herrings — CLUPEIDAE			
Gizzard shad, *Dorosoma cepedianum* (Lesueur)			X
Mooneyes — HIODONTIDAE			
Mooneye, *Hiodon tergisus* Lesueur			X
Mudminnows — UMBRIDAE			
Central mudminnow, *Umbra limi* (Kirtland)	X		
Pikes — ESOCIDAE			
Redfin pickerel, *Esox americanus* Gmelin	X		X
Minnows — CYPRINIDAE			
Stoneroller, *Campostoma anomalum* (Rafinesque)	X	X	X
Goldfish, *Carassius auratus* (Linnaeus)		X	X
Carp, *Cyprinus carpio* (Linnaeus)		X	X
Silverjaw minnow, *Ericymba buccata* Cope	X	X	X
Silvery minnow, *Hybognathus nuchalis* Agassiz	X		X
Bigeye chub, *Hybopsis amblops* (Rafinesque)	X	X	
Hornyhead chub, *Nocomis biguttatus* (Kirtland)	X	X	
River chub, *N. micropogon* (Cope)			X
Golden shiner, *Notemigonus crysoleucas* (Mitchill)	X		X
Popeye shiner, *Notropis ariommus* (Cope)	X		
Emerald shiner, *N. atherinoides* Rafinesque	X		X
River shiner, *N. blennius* (Girard)			X
Bigeye shiner, *N. boops* Gilbert	X	X	X
Striped shiner, *N. chrysocephalus* (Rafinesque)	X	X	X
Common shiner, *N. cornutus* (Mitchill)			X
Rosyface shiner, *N. rubellus* (Agassiz)	X	X	X
Spotfin shiner, *N. spilopterus* (Cope)		X	X
Sand shiner, *N. stramineus* (Cope)	X	X	X
Redfin shiner, *N. umbratilis* (Girard)	X	X	X
Mimic shiner, *N. volucellus* (Cope)		X	X
Steelcolor shiner, *N. whipplei* (Girard)	X		X
Suckermouth minnow, *Phenacobius mirabilis* (Girard)		X	X
Southern redbelly dace, *Phoxinus erythrogaster* (Rafinesque)	X		X
Bluntnose minnow, *Pimephales notatus* (Rafinesque)	X	X	X
Fathead minnow, *P. promelas* Rafinesque			X
Bullhead minnow, *P. vigilax* (Baird & Girard)			X
Blacknose dace, *Rhinichthys atratulus* (Hermann)	X		X
Creek chub, *Semotilus atromaculatus* (Mitchell)	X	X	X
Suckers — CATOSTOMIDAE			
River carpsucker, *Carpiodes carpio*	X		X
Quillback, *C. cyprinus* (Lesueur)		X	X
Highfin carpsucker, *C. velifer* (Rafinesque)			X
White sucker, *Catostomus commersoni* (Lacepede)	X	X	X
Creek chubsucker, *Erimyzon oblongus* (Mitchill)	X	X	X
Northern hog sucker, *Hypentelium nigricans* (Lesueur)	X	X	X
Bigmouth buffalofish, *Ictiobus cyprinellus* (Valenciennes)			X
Spotted sucker, *Minytrema melanops* (Rafinesque)	X		X
Black redhorse, *Moxostoma duquesnei* (Lesueur)		X	X
Golden redhorse, *M. erythrurum* (Rafinesque)		X	X

	1870's	Gerking 1945	Since 1965
Shorthead redhorse, *Moxostoma lepidotum* (Lesueur)	X		
Catfishes — ICTALURIDAE			
Black bullhead, *Ictalurus melas* (Rafinesque)	X		X
Yellow bullhead, *I. natalis* (Lesueur)	X	X	X
Brown bullhead, *I. nebulosus* (Lesueur)	X		X
Channel catfish, *I. punctatus* (Rafinesque)	X		X
Stonecat, *Noturus flavus* Rafinesque	X		
Tadpole madtom, *N. gyrinus* (Mitchill)	X		
Brindled madtom, *N. miurus* Jordan	X		
Flathead catfish, *Pylodictus olivaris* (Rafinesque)	X		X
Pirateperches — APHREDODERIDAE			
Pirateperch, *Aphredoderus sayanus* (Gilliams)		X	
Killifishes — CYPRINODONTIDAE			
Blackstripe topminnow, *Fundulus notatus* (Rafinesque)	X		X
Silversides — ATHERINIDAE			
Brook silversides, *Labidesthes sicculus* (Cope)	X	X	X
Temperate basses — PERCICHTHYIDAE			
Yellow bass, *Morone mississippiensis* Jordan and Eigenmann			X
Bass & Sunfishes — CENTRARCHIDAE			
Rock bass, *Ambloplytes rupestris* (Rafinesque)	X	X	X
Green sunfish, *Lepomis cyanellus* Rafinesque	X		X
Pumpkinseed, *L. gibbosus* (Linnaeus)			X
Warmouth, *L. gulosus* (Cuvier)	X		X
Orange-spotted sunfish, *L. humilis* (Girard)			X
Bluegill, *L. macrochirus* Rafinesque	X	X	X
Longear sunfish, *L. megalotis* (Rafinesque)	X	X	X
Redear sunfish, *L. microlophus* (Gunther)			X
Smallmouth bass, *Micropterus dolomieui* Lacepede	X	X	X
Spotted bass, *M. punctulatus* (Rafinesque)			X
Largemouth bass, *M. salmoides* (Lacepede)	X	X	X
White crappie, *Pomoxis annularis* Rafinesque	X	X	X
Black crappie, *P. nigromaculatus* (Lesueur)	X		X
Perch & Darters — PERCIDAE			
Eastern sand darter, *Ammocrypta pellucida* (Putnam)	X	X	
Greenside darter, *Etheostoma blennioides* Rafinesque	X	X	X
Rainbow darter, *E. caeruleum* Storer	X	X	X
Bluebreast darter, *E. camurum* (Cope)	X		
Fantail darter, *E. flabellare* Rafinesque	X		X
Least darter, *E. microperca* Jordan & Gilbert	X		
Johnny darter, *E. nigrum* Rafinesque	X	X	X
Orangethroat darter, *E. spectabile* (Agassiz)	X		X
Logperch, *Percina caprodes* (Rafinesque)	X	X	X
Channel darter, *P. copelandi* (Jordan)	X		
Gilt darter, *P. evides* (Jordan & Copeland)	X		
Blackside darter, *P. maculata* (Girard)	X		X
Dusky darter, *P. sciera* (Swain)			X
River darter, *P. shumardi* (Girard)	X		
Slenderhead darter, *P. phoxocephala* (Nelson)			X
Yellow perch, *Perca flavescens* (Mitchill)			X
Drums — SCIAENIDAE			
Freshwater drum, *Aplodinotus grunniens* Rafinesque	X		X
Sculpins — COTTIDAE			
Mottled sculpin, *Cottus bairdi* Girard	X	X	X
No. species	63	37	72

SUCKERS - Catostomidae
Quillback, *Carpiodes cyprinus*
Black redhorse, *Moxostoma duquesnei*
Golden redhorse, *M. erythrurum*

PIRATE PERCHES

Pirate perch, *Aphredoderus sayanus*

Christensen (2) undertook studies of the fishes of the White River system, including one electrofishing site at Indianapolis. Christensen's Indianapolis collecting was done August 7 and 8, 1967, from the 16th St. Dam to the New York Street Bridge (T.16N., R.3E., Sect. 34; T.15N., R.3E., Sect. 3). A total of 17 species was reported as indicated in Table 2.

TABLE 2. Fishes from White River, Marion County, Indiana, by author and date.

	Gerking 1945	Christensen 1968	Aquatic Control 1973	Hogan 1975a	Hogan 1975b	WAPORA 1978	IHB 1979-80	Kingsley 1983	ESE 1987
Bowfins — AMIIDAE									
Bowfin					1			1	
Herrings — CLUPEIDAE									
Gizzard shad		91	57	11	88	138	A	135	163
Mooneyes — HIODONTIDAE									
Mooneye							1		
Minnows — CYPRINIDAE									
Stoneroller	x		3		1			13	20
Goldfish		25	8	8	18	7	1	6	27
Carp		36	21	8	21	65	12	127	122
Silverjaw minnow					5			2	52
Silvery minnow				4	1				
Bigeye chub	x								
Golden shiner			3	8	1	17		10	51
Emerald shiner						1			2
River shiner					3				
Bigeye shiner	x								
Striped shiner								5	1
Common shiner			1						1
Spotfin shiner				4	1	6		218	867
Sand shiner				2		3		8	1
Redfin shiner						1			6
Mimic shiner			1						1
Steelcolor shiner			14	5	2				6
Suckermouth minnow	x				2			8	7
Bluntnose minnow	x		4	5	75	87		186	467
Fathead minnow				1	2	1			6
Bullhead minnow					4				1
Blacknose dace			3						
Creek chub				1	14			9	3
Sucker — CATOSTOMIDAE									
River carpsucker		20		4			2		19
Quillback		5		4		1	4	98	1
Highfin carpsucker		6	33	7	1				1
White sucker			1	3	4	7		26	26
Creek chubsucker	x								
Northern hog sucker			1	1				11	2
Bigmouth buffalofish								3	3
Spotted sucker			2			1	6	22	56
Black redhorse	x		2	1					
Golden redhorse		17						12	8
Catfishes — ICTALURIDAE									
Black bullhead		5		1					12
Yellow bullhead						1		3	8
Brown bullhead		4							
Channel catfish			1			2		120	5
Flathead catfish								7	
Silversides — ATHERINIDAE									
Brook silversides						1	1	1	11
Temperate basses — PERCICHTHYIDAE									
Yellow bass									3

	Gerking 1945	Christensen 1968	Aquatic Control 1973	Hogan 1975a	Hogan 1975b	WAPORA 1978	IHB 1979-80	Kingsley 1983	ESE 1987
Bass & Sunfishes — CENTRARCHIDAE									
Rock bass								6	1
Green sunfish		7	7	6	53	133	5	29	150
Pumpkinseed		29							9
Orange-spotted sunfish		3	1	5	26	17		57	67
Bluegill		13	5	13	53	31	37	38	226
Longear sunfish	x	91	99	12	52	282	33	617	466
Redear sunfish				1		1			
Smallmouth bass								45	1
Spotted bass		4				43		3	66
Largemouth bass		68	12	10	1	13	20	121	173
White crappie		8	2	9	4		17	34	96
Black crappie			4				2	23	37
Perch & Darters — PERCIDAE									
Greenside darter									
Rainbow darter								1	
Fantail darter									
Logperch	x								
Slenderhead darter								1	
Drums — SCIAENIDAE									
Freshwater drum									1
TOTAL	9	17	23	25	24	23	14	35	44

A = Abundant

Five of these species had not been previously reported from Marion County, bringing the total number of species known from the county to 77. The five are:

Gizzard shad, *Dorosoma cepedianum*

Highfin carpsucker, *Carpiodes velifer*

Orange-spotted sunfish, *Lepomis humilis*

Pumpkinseed, *L. gibbosus*

Spotted bass, *Micropterus punctulatus*

The gizzard shad, the carpsucker, the spotted bass, and the orange-spotted sunfish are all species now commonly found in larger silty streams, perhaps directly or, more likely, indirectly because of siltation (many other species appear to decrease with siltation). Also, the spotted bass was often overlooked in earlier studies. The pumpkinseed is a species of far northern Indiana. Records from Gerking were almost entirely from north of the Wabash River. Presumably the Pumpkinseed has been introduced at Indianapolis. The brown bullhead is primarily a species of northern and southern Indiana, with a rather distinct break in the central part of the state (7).

The number of species of fish generally decreases, but the number of individuals of tolerant species generally increases in partially polluted streams. Christensen (2) found more fish species at the Indianapolis collecting site than at any other site he studied on the west fork of the White River. This site is in the central part of the city, before most of the pollutants enter. However, at Waverly, in Morgan County, 16 miles below Indianapolis, only carp were taken, and even they were in poor condition.

Twenty-five miles below Indianapolis, at Exchange, Morgan Co., four species were taken, with the number per hour given in parentheses: carp (39.96), goldfish (1.76), green sunfish (1.76), longear sunfish (8.80). At Martinsville, Morgan Co., about 30 miles below Indianapolis, the number of fish species taken was twelve.

Aquatic Control, Inc. (1) made four collections near the Perry K and three near the E.W. Stout generating plants of the Indianapols Power and Light Company at Indianapolis. Perry K is about 0.3 mi south of Washington St. The four collections

TABLE 3. Fishes from Eagle Creek, Marion County, Indiana. Data presented as presence (x) or the actual number taken, when available.

		Hogan 1975a					
		Near					
		Sewage	Washington St.			Fisher & Gammon	
	Gerking	Disposal	Big	Little	Kingsley	School	Fishback
	1945	Plant	Eagle	Eagle	1983	Branch	Creek
Herrings — CLUPEIDAE							
Gizzard Shad	x					5	
Minnows — CYPRINIDAE							
Stoneroller	x				36	121	528
Goldfish		x					
Carp						1	
Silverjaw minnow			x	x	1	9	4
Bigeye chub	x						
Hornyhead chub	x						
River chub						1	
Bigeye shiner	x						
Striped shiner						7	131
Common shiner	x						
Rosyface shiner	x						
Spotfin shiner	x		x				1
Sand shiner					2		
Redfin	x					1	36
Suckermouth minnow	x				1		2
Bluntnose minnow	x		x	x	22	30	406
Blacknose dace	x		x		1		
Creek chub	x				7	239	379
Sucker — CATOSTOMIDAE							
White sucker					4	48	110
Creek chubsucker							1
N. hog sucker			x		39		
Black redhorse					4		
Catfishes — ICTALURIDAE							
Black bullhead						53	
Yellow bullhead	x				15		2
Silversides — ATHERINIDAE							
Brook silversides					3	1	
Bass & Sunfishes — CENTRARCHIDAE							
Rock bass							8
Green sunfish					18	7	55
Orange spotted sunfish			x				
Bluegill				x	9	31	61
Longear sunfish	x				10	12	3
Spotted bass						9	8
Largemouth bass					7	12	4
Smallmouth bass	x						
White crappie		x	x			1	
Perch & Darters — PERCIDAE							
Greenside darter	x				1	2	
Rainbow darter	x		15		2		
Fantail darter					2		
Johnny darter						21	100
Orangethroat darter						98	128
Logperch	x					64	56
Blackside darter							9
Dusky darter							5
Sculpins — COTTIDAE							
Mottled sculpin					15	152	
	19	2	8	3	20	23	22

TABLE 4. Collections from Fall Creek, Marion County, Indiana. Data presented as presence (x) or the actual number taken, when available.

		WAPORA, Hogan		Kingsley 1983			
	Gerking	1975a		Indiana	30th	56th	79th
	1945	Mouth	I-65 N	Ave.	St.	St.	St.
Herrings — CLUPEIDAE							
Gizzard shad				5	3	20	14
Minnows — CYPRINIDAE							
Stoneroller	x			1			
Goldfish	x	x			5		
Carp		x		10	33	9	
Silverjaw minnow	x						
Bigeye chub	x(?)						
Hornyhead chub	x						
Golden shiner					3		
Striped shiner	x						
Rosyface shiner	x(?)						
Spotfin shiner	x	x		60	7	2	2
Sand shiner	x			64			
Redfin shiner	x						
Mimic shiner	x						
Suckermouth minnow	x			9			
Bluntnose minnow	x	x		56	75	8	
Fathead minnow						1	
Suckers — CATOSTOMIDAE							
Quillback	x			8			
White sucker	x			1	3	5	
Northern hog sucker	x			7		9	
Spotted sucker					10	4	1
Golden redhorse	x			2		19	
Catfishes — ICTALURIDAE							
Yellow bullhead					1		
Channel catfish				8		1	
Bass & Sunfishes — CENTRARCHIDAE							
Rock bass	x				1	4	
Green sunfish			x		1		5
Orangespotted sunfish			x				
Bluegill	x	x	x		1		5
Longear sunfish	x	x	x	10	39	37	23
Smallmouth bass	x			4		12	
Spotted bass					1		
Largemouth bass	x			2	13	1	3
White crappie	x			1		3	
Black crappie				1			
Perch & Darters — PERCIDAE							
Eastern sand darter	x						
Greenside darter	x						
N. Rainbow darter	x						
Johnny darter	x						
Yellow perch							1
Sculpins — COTTIDAE							
Mottled sculpin	x						
	28	6	4	17	15	15	8

were by electrofisher on September 27, 1973. Data from the four are summarized in Table 2. Longear sunfish, gizzard shad, highfin carpsucker and carp were the most abundant species. The Stout plant is above a dam about 0.7 miles below the mouth of Eagle Creek, and below the Indianapolis Sewage Disposal plant, from which it received water with low dissolved oxygen (3.8 to 5.6 mg/liter here as opposed to 7.2

TABLE 5. Fishes taken in several smaller creeks of Marion County in Kingsley (1983).

	Crooked Creek	Dollar Hide	Lick Creek	Little Buck	Williams	Howland Ditch
Minnows — CYPRINIDAE						
Stoneroller	8	66	9	29	3206	
Carp	5					1
Silverjaw minnow		4	14	13	335	
Emerald shiner	1				24	
Striped shiner		1			1	
Spotfin shiner					4	2
Sand shiner					201	
Redfin shiner					3	
Suckermouth minnow					199	
S. redbelly dace		5				
Bluntnose minnow	1	1	20	1	310	1
Blacknose dace		23		34	35	
Creek chub	6	34	71	27	490	
Suckers — CASTOSTOMIDAE						
White sucker		5	16	2	272	
Creek chubsucker	2				1	
N. hog sucker	13				89	
Catfishes — ICTALURIDAE						
Black bullhead	4					
Yellow bullhead			4		6	1
Bass & Sunfishes — CENTRARCHIDAE						
Rock bass					1	
Green sunfish	43	2	13		1	19
Pumpkinseed						1
Warmouth	1					3
Bluegill	7		3			16
Longear sunfish	5					13
Smallmouth bass					2	
Spotted bass			2		2	
Largemouth bass	3				1	11
Perch & Darters — PERCIDAE						
Orangethroat darter		5			21	
TOTAL SPECIES	13	10	9	6	21	10

to 8.8 at Perry K). Only 39 fish of five species were taken at the three sites near the Stout plant. They were: bluntnose minnow, *Pimephales notatus* (19 individuals), carp, *Cyprinus carpio* (16), green sunfish, *Lepomis cyanellus* (2), bluegill, *L. macrochirus* (1), and golden shiner, *Notemigonus crysoleucas* (1). The low numbers and low diversity were assumed to be due primarily to the sewage plant effluent. A total of 23 species was taken during the Aquatic Control studies (Table 2), all but one previously reported from Marion County. The one was the common shiner, *Notropis cornutus*, bringing the fish to a total of 78 species known from Marion County.

WAPORA, Inc., carried out electrofishing and seining June 2 to 6, 1975 (8) in the White River (Table 2). Eleven zones were sampled in the river, zone 1 above the mouth of Lick Creek; 2 & 3 east of Harding St.; 4 north of Raymond St.; 5 between Morris St. and Interstate 70; 6, 7 & 8 north of Oliver Ave.; 9 just north of New York St.; and 10 and 11 near 16th St. Seining was carried out at 7 sites, 2 near electrofishing zone 1, 2 near zones 2 & 3, 1 near zone 4, 1 at zone 5 and 1 near zones 6-8.

The total number of species taken by electrofishing and seining between the White River from north of I-465 at the south edge of the city north to about 16th

TABLE 6. Fishes recently collected in Indianapolis Water Company Canal (Hlavec, unpublished; Kingsley, 1983) and Pleasant Run (1983). Data presented as presence (x) or the actual number taken, when available.

	Indianapolis Water Co. Canal						Pleasant Run	
	Hlavec, unpublished				Kingsley 1983		Kingsley, 1983	
	Broad Ripple	Butler Univ.	35th St.	24th St.	Burdsal Pkwy.	College Ave.	Bluff Road	English Ave.
Herrings — CLUPEIDAE								
Gizzard shad					3	3		
Pikes — ESOCIDAE								
Redfin pickerel		x						
Minnows — CYPRINIDAE								
Stoneroller							359	
Goldfish						1		
Carp						4	6	
Silverjaw minnow					1		3082	
Golden shiner	x				3	4		
Emerald shiner							3	
Spotfin shiner						8	7	
Sand shiner							744	
Mimic shiner						1		
Suckermouth minnow						3	1746	
Bluntnose minnow						18	1904	
Fathead minnow							340	3
Blacknose dace							220	
Creek chub							73	
Suckers — CATOSTOMIDAE								
Quillback						1	7	
White sucker							9	
Spotted sucker					39	48		
Golden redhorse						3		
Catfishes — ICTALURIDAE								
Yellow bullhead	x		x	x			1	
Channel catfish					4	21		
Flathead catfish					4			
Killifishes — CYPRINODONTIDAE								
Blackstripe topminnow			x	x	1			
Silversides — ATHERINIDAE								
Brook silverside	x							
Bass & Sunfishes — CENTRARCHIDAE								
Rock bass	x	x	x	x	2	1		
Green sunfish	x	x	x	x			30	
Orange-spotted sunfish							7	
Bluegill	x	x			3	2	1	
Longear sunfish	x	x	x	x	11	22		
Smallmouth bass						2		
Largemouth bass	x		x	x	3	6		
White crappie	x				1	2		
Black crappie						1		
Perch & Darters — PERCIDAE								
Johnny darter	x					1		
No. species	10	5	6	6	12	20	17	1

St. was 25, 23 by electrofishing, 19 by seining (Table 2). Three species were taken by seining, not by electrofishing: the fathead minnow, the sand shiner, and the creek club. Additional electrofishing was carried out at 4 sites in Eagle Creek (Table 3), at the Sewage Disposal Plant (just north of its confluence with the White River), just above the sewage disposal plant, in Eagle Creek, and in Little Eagle Creek south

of Washington St. just before the two streams converge. Two electrofishing sites were established in Pleasant Run, one at the mouth of the creek and one just south of Raymond St. In Fall Creek, there was one site at the mouth and one at the dam near Meridian St. Excluding *Ictiobus* identified only to genus, three new species for Marion County were taken by Hogan (8): the fathead minnow, *Pimephales promelas*, the bullhead minnow, *Pimephales vigilax*, and the redear sunfish, *Lepomis microlophus*.

Two species reported by Hogan (8), the longnose dace, *Rhinichthys cateractae*, and the bigmouth shiner, *Notropis dorsalis*, are likely misidentifications. The one previous record for the state of *R. cateractae* is from the St. Joseph River, Elkhart County (7). The blacknose dace, *R. atratulus*, had been previously reported from Marion County (1), and is fairly common in Indiana, but was not reported by WAPORA. We suspect WAPORA misidentified *R. atratulus* as *R. cateractae*, and have included the record here as *R. atratulus*. The bigmouth shiner, *Notropis dorsalis*, has not been previously taken in Indiana and is reported by Gerking (7) as of doubtful occurrence. Specimens of this species should be reexamined; until they can be verified, we have chosen to omit them from further consideration.

A second WAPORA study (9) was carried out for the Indianapolis Power and Light Company (IPALCO) in the degraded section of the river below the Indianapolis Sewage Treatment Plant and in the area of the IPALCO E.W. Stout Plant in southwestern Indianapolis, north of I-465 and west of Harding Street. Sampling occurred in 7 electrofishing zones and 7 seining zones, some the same zones used by Hogan (8).

Surprisingly, a total of 24 species of fish was taken in this area (Table 2), including 2 species not taken previously from Marion County, *Notropis blennius*, the river shiner, and *Amia calva*, the bowfin. Thirteen species were taken above the Sewage Plant, 11 were taken between the Sewage Plant and the E.W. Stout dam, and 19 species were taken below the dam. However, the numbers of individuals were much reduced in the outflow area from the sewage plant; 1.7 fish being taken per 10 minute period here by electrofishing, as compared to 19.0 upstream above the sewage plant, and 15.2 downstream below the E.W. Stout dam.

More recently, WAPORA, Inc., carried out an additional study of water quality and fish populations in the White River (11), from Indianapolis to Petersburg, Indiana, about 200 river miles. WAPORA established a total of 27 sampling zones, 7 of them in Marion County. Each of these was sampled twice, once during each of the periods August 23-30 and October 21-28, 1977. Sampling consisted of 10-30, usually 20 minutes effort.

Zones were:

Zone	River Mile*	Location	*Dissolved Oxygen (mg/g) and Ammonia (mg/l)* Aug. 23-24 O_2	Aug. 23-24 NH_3	Oct. 21-22 O_2	Oct. 21-22 NH_3
1	238.0	Above Fall Creek	8.4	0.1	9.4	0.2
2	236.2	Above Perry K	7.2	0.3	8.8	0.3
3	235.7	Below Kentucky St.	8.0	0.3	9.6	0.3
4	233.7	Above Harding St. Bridge	6.2	0.6	8.4	0.5
5	232.0	Just above Lick Creek	4.0	6.1	7.3	2.9
6	231.5	Just below Lick Creek	4.2	4.0	8.5	2.9
7	227.0	Just below Buck Creek	4.0	10.9	4.7	6.5

*River mile designation is for upstream end of sampling zone, and is from US Corps of Engineers 1931 charts for the White River.

A total of 23 species of fish was taken during this work (Table 2), but all had been previously taken in Marion County.

In 1978, Roger Hlavec of Marian College, Indianapolis, collected fishes from the Indianapolis Waterway Canal (unpublished). Unfortunately, most of the minnows have not yet been identified. Hlavec collected at four locations on the canal (Table 6), and collected twelve species of fish.

Three fish collections were made by electrofishing in the White River by the Indiana State Board of Health, Division of Water Pollution Control (unpublished). The first two were near Broad Ripple Park in northern Marion County on 6 Sept. 1979 and 8 Sept. 1980. In these two samples, only fish collected for analysis were tabulated. These consisted of five each of largemouth bass, *Micropterus salmoides*, longear sunfish, *Lepomis megalotis*, and carp, *Cyprinus carpio*, in the first collection, and spotted sucker, *Minytrema megalops*, longear sunfish, largemouth bass, and carp in the second collection. Gizzard shad were also listed as abundant at this station in 1979. The third collection was made between Michigan and Washington Streets on Dec. 1, 1980. Fishes taken were gizzard shad (2), mooneye (1), goldfish (1), carp (2), river carpsucker (2), quillback (4), spotted sucker (1), brook silversides (1), green sunfish (5), longear sunfish (23), bluegill (37), largemouth bass (10), white crappie (17), and black crappie (2). This collection adds one additional species, the mooneye, *Hiodon tergisus*, not previously reported from the county.

A three-year study of agricultural effects included studies of fishes at several localities in central Indiana, including two Marion County localities, both tributaries of Eagle Creek (Fisher and Gammon, 5). One was Fishback Creek below and under Wilson Road Bridge, 1.0 mi west of Traders Point. The second locality was locality E-1 from Hendricks County. The collection was from School Branch at County Line Road Bridge, Hendricks/Marion Counties, and some of the collecting was in Marion County; thus this collection is included also. Fisher and Gammon took a total of 30 species (Table 3), including 23 at School Branch and 22 at Fishback Creek. Two of the species were new to Marion County, the river chub, *Nocomis micropogon*, and the dusky darter, *Percina sciera*.

Kingsley (10) undertook an electrofishing survey in Marion County to accumulate further information on the fishes. Thirteen sampling sections were established on the main stem of the White River, 15 on the tributaries, Eagle Creek (3), Fall Creek (4), Pleasant Run (2), Lick, Little Buck, Dollar Hide, Crooked, and Williams Creeks (1 each), one on Howland Ditch, and 2 on the Indianapolis Water Company Canal (2). Different methods were used depending on conditions at the site, but electrofishing, gill nets, trap nets and seines were used. A total of 17,378 fishes of 54 species was collected (Tables 2-6). Four species not previously taken in Marion County were included, the bigmouth buffalofish, *Ictiobus cyprinellus*, the yellow bass, *Morone mississippiensis*, the slenderhead darter, *Percina phoxocephala*, and the yellow perch, *Perca flavescens*. Four additional ones, the flathead catfish, the southern redbelly dace, the fantail darter, and the warmouth had not been taken in this century from Marion County.

Thirty-five species were taken in the White River (Table 2), six species in Little Buck Creek, 13 in Crooked Creek, 10 in Dollar Hide Creek, 9 in Lick Creek, 21 in Williams Cree, and 10 in Howland Ditch, each in one collection (Table 5).

In 3 collections in Eagle Creek (Table 3), Kingsley took 20 species and 199 individuals of fish, including 2 fantail darters, 2 rainbow darters, 1 greenside darter, and 15 mottled sculpins. Two collections were made on Pleasant Run, with one at Bluff Road yielding 17 species and 8539 individuals (Table 6). One at English Avenue yielded 3 fathead minnows only. Two collections in the Indianapolis Water Company Canal yielded a total of 227 individuals including 23 species. Twenty-five species were taken in 4 collections in Fall Creek (Table 4).

Environmental Science and Engineering, Inc. has carried out additional sampling of the lower 200 miles of the White River. This work involved electrofishing and seining and included collections on the White River in Marion County near the Perry K and E.W. Stout Indianapolis Power and Light Company dams. Forty-four species were taken during this work (Table 2), but all had been previously taken.

This brings to 90 the number of species reported from Marion County (Table 1).

Species from Various Streams in Marion County

Much of the collecting in Marion County has been in the White River. The fish species taken there recently (since 1940) total 61 species (Table 2). One collection was made by Gerking (6) in the White River, about 8 miles north of Indianapolis. In it were found only 9 species. However, other workers have reported numbers of species varying up to 44 (9) with the number of species much influenced by the amount and type of collecting.

One might think that the number of species would increase to the north in the White River in Marion County, in relation to decreased pollutant load of the river. Individual collections in the White River were listed in approximate order from south to north as follows. (We have combined some of these data such as seining and electrofishing collections in the same area or collections on opposite sides of the river. Numbers and letters refer to original collection designations.)

		No.	*species*
Kingsley #1	Johnson Co. Line		5
Kingsley #2	Southport Road		13
WAPORA 1978#7	Below Southport sewage treatment plant		2
Kingsley#3	I-465		17
WAPORA 1978 #6	Below Lick Creek		7
ESE 1987	E.W. Stout vicinity		34
Hogan 1975b	E.W. Stout plant		24
Hogan 1978 #4	Below E.W. Stout Dam		11
Hogan 1978 #5	Above E.W. Stout Dam		11
Aquatic Control	3 collections in Lick Creek area near E.W. Stout Dam	EWS 1	4
		EWS 2	1 (carp)
		EWS 3	1 (carp
Hogan 1975a Ha1	Area near E.W. Stout Dam		14
Hogan 1975a Ha2	Area near E.W. Stout Sam		12
Kingsley #4	Harding Street		19
Hogan 1975a Ha3	At Raymond Street		16
Hogan 1975a Ha4	At I-70		18
Hogan 1978 #4	Upstream from Harding St.		11
Aquatic Control	4 collections in area of Perry K Power Plant	PK 1	10
		PK 2	8
		PK 3	11
		PK 4	18
Hogan 1975a Ha5	Below Perry K		15
Hogan 1975a Ha6	Above Perry K		8
Hogan 1978 #3	Downstream from Kentucky Ave.		11
Hogan 1978 #2	Upstream from Perry K		13
ESE 1987	Perry K		39
Kingsley #5	Washington-Michigan St.		17
Hogan 1975a Ha7	New York Ave.		9

		No.	*species*
Christiansen	16th St. to New York St.		17
Hogan 1975a Ha8	16 St. to New York St.		16
Hogan 1978-1	Upstream from Fall Creek		10
Kingsley #6	Lake Indy, lower end		17
Kingsley #7	Lake Indy, upper end		14
Kingsley #8	Northwestern Avenue		18
Kingsley #9	Kessler Boulevard		13
Kingsley #10	Broadripple, lower end		15
Kingsley #11	Broadripple, upper end		14
Kingsley #12	79th St.		15
Kingsley #13	96th St.		14

There is no steady increase of fish species to the north. Several factors affect the individual collection data, type and amount of time of collection, amount of pollution, and type of habitat and habitat variation present. However, the average number of species per collection did tend to increase to the north. The four collections in the most southerly part of the county averaged 9.3 species per collection. Progressing northward similar values were $\bar{x}$ = 12.4 (12 collections in vicinity of E.W. Stout Generating Station), 17.0 (2 collections, between E.W. Stout and Perry K), 12.2 in 6 collections in vicinity of Perry K, 16.3 between Washington St. and U.S. 36, and 19.8 north of U.S. 36. Thus numbers were lower in the extreme southern part of the county and near the two generating plants, but generally higher to the north.

Data collected from Eagle Creek are summarized in Table 3. A total of 44 species has been taken, with individual collections varying from 2 to 23 species. In Eagle Creek there are few fish downstream near the Sewage Disposal Plant, but upstream 20 species were collected by Kingsley (10) and 30 were taken by Fisher and Gammon (5).

Fall Creek was sampled at seven sites (7, 8, 10; Table 4). The site sampled by Gerking was apparently near I-465, whereas one of those of WAPORA was at the mouth; the second was in central Indianapolis at about I-65 North. Only six species were taken at the mouth of Fall Creek, and four at I-65 North by WAPORA, whereas 27 species were taken upstream by Gerking, and 24 by Fisher and Gammon (5). Thus lower Fall Creek harbors few species, but upstream Fall Creek still harbors a number of species.

Two sites in Pleasant Run Creek were sampled by WAPORA, one at the mouth and one just south of Raymond Street. Much of this stream is heavily polluted and no fish were taken at either point. However, Kingsley (10, Table 6) sampled upstream in Pleasant Run and collected 17 species in addition to 3 carp at a downstream locality.

Collections were made by Kingsley (10) in six of the other smaller streams of Marion County. The number of species per collection in these streams ranged from 6 to 21. One of these was in Little Buck Creek, a creek in which Gerking (7) sampled. Gerking collected 15 species, as follows: Stoneroller, silverjaw minnow, striped shiner, spotfin shiner, sand shiner, redfin shiner, suckermouth minnow, bluntnose minnow, white sucker, creek chubsucker, northern hogsucker, longear sunfish, largemouth bass, greenside darter, and rainbow darter. Kingsley collected 6 species in this stream. Williams Creek, flowing into the White River in the north central part of Marion County, harbored the most diverse fish community of the smaller streams, with 21 species being taken.

Hlavec (unpublished) and Kingsley (10) sampled fishes of the Indianapolis Waterway Canal. Twelve species were identified, but many of the minnows taken by Hlavec

are as yet undetermined. Kingsley took a total of 22 species at the two sites from which he collected.

Discussion

Fourteen of the 90 species of fishes known from Marion County were taken in the 1870's, but not since. They are the silver lamprey, *Ichthyomyzon unicuspis*, paddlefish, *Polyodon spathula*, longnose gar, *Lepisosteus osseus*, central mudminnow, *Umbra limi*, popeye shiner, *Notropis ariommus*, shorthead redhorse, *Moxostoma macrolepidotum*, stonecat, *Noturus flavus*, tadpole madtom, *N. gyrinus*, brindled madtom, *N. miurus*, bluebreast darter, *Etheostoma camurum**, least darter, *E. microperca**, channel darter, *Percina copelandi**, gilt darter, *P. evides**, and river darter, *P. shumardi**.

Three additional species were taken in the 1870's and by Gerking (7) but not since. They are: bigeye chub, *Hybopsis amblops*; horny head chub, *Nocomis biguttatus*; and the eastern sand darter, *Ammocrypta pellucida*; and one species, the pirateperch, *Aphredoderus sayanus*, was taken only by Gerking (7). This brings to 18 the species that have not been taken in recent years (1965 to present). Some of these are endangered or threatened in Indiana (those marked with an asterisk). It seems unlikely that all of these species have become extirpated since 1945. It would seem likely that further sampling of the larger streams would turn up at least the silver lamprey and longnose gar, and intensive sampling with seines in relatively unpolluted upstream areas might turn up some of the other species.

This leaves 72 species of fish that have been collected in Marion County since 1965.

Eighteen species were not reported until the more recent studies. They are: bowfin, *Amia calva*; gizzard shad, *Dorosoma cepedianum*, mooneye, *Hiodon tergisus*; river chub, *Nocomis micropogon*, river shiner, *Notropis blennius*; common shiner, *Notropis cornutus*; fathead minnow, *Pimephales promelas*; bullhead minnow, *P. vigilax*; highfin carpsucker, *Carpiodes velifer*; bigmouth buffalofish, *Ictiobus cyprinellus*; yellow bass, *Morone mississippiensis*; pumpkinseed, *Lepomis gibbosus*; orange-spotted sunfish, *L. humilis*; redear sunfish, *L. microlophus*; spotted bass, *Micropterus punctulatus*; dusky darter, *Percina sciera*; slenderhead darter, *Percina phoxocephala*; and yellow perch, *Perca flavescens*.

Some of these may simply have been overlooked in earlier studies. The redear sunfish and pumpkinseed may have been brought in by man. The redear sunfish, in particular, is often stocked. Some of these species often thrive in silty water situations, and may have invaded or at least increased their numbers considerably with increased siltation and other forms of pollution. The decrease in numbers or disappearance of species partial to clear water such as darters and madtoms could further enhance the success of these forms.

It is apparent that the streams in the southwest section of Indianapolis are quite heavily polluted and silted; and that this has decreased or eliminated fish from many portions. However, it is also evident that there are places even here where fish still exist, sometimes with fair diversity. It is also clear that many of the upstream areas of Marion County harbor numerous fish of many species.

We suspect that more fish species will be found with additional collecting such as that done in Vigo County where 108 species are known (13). An intensive survey of Marion County, with concentration on the smaller, relatively unpolluted upstream areas, would give us a more complete knowledge of its fishes.

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INSTRUCTIONS FOR CONTRIBUTORS

At its meeting on June 8, 1988, members of the Publications Committee unanimously adopted the current edition of the *CBE Style Manual*, published by the Council of Biology Editors, Inc., as the official guide for authors of the Academy's publications.

Correct citation for the current edition is:

CBE Style Manual Committee: CBE style manual: a guide for authors, editors, and publishers in the biological sciences (5th ed., revised and expanded). Bethesda, MD: Council of Biology Editors, Inc., 1983.

PREPARATION OF MANUSCRIPTS FOR PUBLICATION IN THE PROCEEDINGS OF THE INDIANA ACADEMY OF SCIENCE

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ABSTRACTS. The abstract should be a meaningful summary of the significant facts contained in the paper or Brief Note and should be appropriate for the published article (an Abstract proposal specifically for oral presentations may need to be modified). It should be sufficient within itself, presenting the main conclusions of the paper or Note and any new methods or procedures critical to the results presented in the paper. It should be brief (not over 250 words) but complete sentences should be used to promote comprehension and clarity. Words such as "is presented," "is explained," or "is discussed" should be avoided. Author abstracts from the

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Specific examples may be found in the *Newsletter* of The Indiana Academy of Science, No. 58, October 1988, p. 13-14.

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